A systematic review of the effect of rearing substrate composition on the fatty acid profile of black soldier fly larvae (*Hermetia illucens*)

by

Elizabeth Yvonne Gleeson

Thesis presented in fulfilment of the requirements for the degree of

Master of Science in Animal Science in the Faculty of AgriSciences

at

Stellenbosch University

Supervisor: Dr. Elsje Pieterse

December 2021

Stellenbosch University https://scholar.sun.ac.za

DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof, that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: September 2021

Copyright © 2021 Stellenbosch University
All rights reserved

Stellenbosch University https://scholar.sun.ac.za

ABSTRACT

As the agricultural sector is faced with providing sufficient nutritious and affordable food for the global population, the need for more sustainable agricultural practices is highlighted. One of the ways to improve the sustainability of the overburdened agricultural sector is the use of feed ingredients that have a reduced impact on the environment, while still optimising production. The black soldier fly (*Hermetia illucens*) has been well researched as a protein source for both animal feed and human consumption, as it could augment more traditional protein sources such as soybean meal, which has a large environmental impact. The black soldier fly larvae are also high in lipids and have the potential to be utilized as a source of energy in the form of fatty acids. The use of black soldier fly larvae as a lipid source is however less well researched. Studies have shown that the larval fatty acid profile is affected by multiple factors including: nutrition, age and environmental conditions. In recent years, numerous studies have investigated the effect that the rearing substrate composition has on the larval fatty acid profile. The systematic review performed as part of this study aimed to consolidate and compare the findings of published research regarding this effect. Meta-analyses were incorporated into the systematic review to determine to which extent the concentrations of the individual fatty acids in the larval fatty acid profile are affected by the rearing substrate composition. The results suggested that black soldier fly larvae's importance as a source of lipids and the effect that nutrition has on their fatty acid profile is recognised by researchers globally and that the interest in the topic has increased in recent years. It did however also show that there is a lack of standardisation in terms of larval rearing trial methodologies, which has a potential impact on the larval fatty acid profile. The findings of the systematic review clarified to which extent the larval fatty acid profile can be changed through changes in the rearing substrate composition. The meta-analysis results indicated that the concentrations of many of the fatty acids are significantly affected by the rearing substrate composition. The largest effect was found for the lauric acid concentration. Subsequently the level of saturation, in other words the total amount of saturated-, monounsaturated- and polyunsaturated fatty acids, are also significantly affected by the rearing substrate composition. This information could contribute to more dynamic larval nutrition thereby tailoring the larval fatty acid profile to its intended purpose, whether that be animal feed, human consumption or another purpose such as biofuel production.

Stellenbosch University https://scholar.sun.ac.za

OPSOMMING

Soos wat die landbou sektor streef om voldoende voedsame en duursame voedsel te voorsien aan die populasie, word die nood vir meer volhoubare landbou praktyke beklemtoon. Een van die maniere hoe volhoubaarheid van ‘n alreeds oorbelaste landbou sektor kan verbeter, is deur die gebruik van voerbestandele wat ‘n verminderde impak op die omgewing het en steeds produksie optimaliseer. Die venstervlieg (*Hermetia illucens*) is ‘n insekspesie wat al baie nagevors is as ‘n proteïenbron vir beide voer en menslike gebruik. Dit verteenwoordig ‘n aanvulling vir meer tradisionele proteïenbronne soos soja, wat ‘n groot impak op die omgewing het. Die venstervlieg larwes bevat ook baie lipiedes en het die potensiaal om gebruik te word as a bron van energie in die vorm van vetsure. Daar is egter minder navorsing gedoen op die gebruik van venstervlieg larwes as a lipied bron. Studies het al gewys that die larwes se vetsuurprofiel geaffekteer word deur meerdere faktore, insluitende voeding, ouderdom en omgewingstoestande. In die afgelope paar jaar is daar meer studies gedoen wat gefokus het op die effek van die groeisubstraatkomposisie op die larwes se vetsuur profiel. Die stelselmatige literatuurstudie wat deel gevorm het van hierdie studie het daarop gefokus om die bevindings van gepubliseerde navorsing te konsolideer en vergelyk. Meta-analises was ook geïnkorporeer om te bepaal tot watter mate die konsentrasies van die individuele vetsure beïnvloed word deur die groei susbtraat-komposisie. Die resultate het aangedui dat daar wêreldwye belangstelling is in die venstervlieg larwes se potensiaal as ’n bron van lipiede en die effek wat voeding het op hulle vetsuur profiel. Dit het egter ook daarop aangedui dat daar ‘n gebrek aan standaardisering is in terme van die metodologieë wat geïmplimenteer word tydens die groeiproewe. Hierdie metogologiese variasies kan ook ‘n invloed hê op die larwes se vetsuur-profiel. Die stelselmatige literatuurstudie se bevindings verduidelik hoe die vetsuur-profiel van die larwes verander kan word deur wysigings aan te bring in the groeisusbtraatkomposisie. The meta-analise se resultate het aangedui dat die konsentrasie van meeste van die individuële vetsure beïnvloed word deur die groeisubstraatkomposisie. Die grootste effek was gesien by lauriensuur. Gevolglik is dit ook moontlik om die vetsuur-profiel se vlak van versadiging, met ander woorde die konsentrasie van die versadigde, mono-onversadigde en poli-onversadigde vetsure, te beïnvloed deur wysigings aan te bring in die groeisubstraat. Hierdie inligting kan hopelik bydra tot meer dinamiese larwevoeding. Daardeur is dit dan moontlik om die larwes se vetsuurprofiel aan te pas vir hulle uiteindelike doel, al is dit voer, kos of miskien ‘n ander doel soos biobrandstofproduksie.

Stellenbosch University https://scholar.sun.ac.za

DEDICATION

This thesis is dedicated to the loving memory of my grandfather, Prof Daan Pienaar, who was my first teacher and my greatest supporter.

(1934-2020)

Stellenbosch University https://scholar.sun.ac.za

ACKNOWLEDGEMENTS

I wish to express my gratitude and appreciation to the following institutions for funding my research and supporting my studies:

- The South African Research Chairs Initiative of the Department of Science and Technology and the National Research Foundation of South Africa (UID: 84633). Any opinions, findings and conclusions or recommendations expressed in this material is that of the author(s) and the National Research Foundation does not accept any liability in this regards.
- The Postgraduate Office at Stellenbosch University for their support during my postgraduate studies.
- The Erasmus+ EU programme for giving me the opportunity to complete eight months of my studies at *Università di Padova*, Italy.

I would like to thank the following people for their support and assistance during my postgraduate studies:

- My supervisor Dr Elsje Pieterse for her guidance and support, I could not have asked for a better supervisor.
- Prof Antonella Dalle Zotte, Dr Marco Cullere and the rest of the research team at *Università di Padova* for the opportunity to develop my research abilities and the support during an unforeseen difficult time.
- Prof Ilse Fraeye and the team at *Katholieke Universiteit Leuven* for allowing me work with them for five month during a difficult time in Europe and giving me the opportunity to learn a great deal.
- Prof Daan Nel at the Centre for Statistical Consultation at Stellenbosch university for assisting me with the meta-analyses.
- Elizabeth Will-Mollard at the Stellenbosch University library for her assistance with the planning of the systematic review.
- Dr Danie Stander for his assistance with the language and editing.
- My mother, Elzabé Heyns for technical editing and support.

Stellenbosch University https://scholar.sun.ac.za

I would also like to thank a few special people:

- My aunts Nanna and Corlien, who's support and love made it possible for me to pursue a career in science.
- My grandparents Betta and Prof Daan Pienaar, who are the reason I decided to study science in the first place and without who's love I would not be in this fortunate position.
- My partner Simeon, who made countless cups of coffee and tea in the middle of the night to keep me motivated, I love you.

Stellenbosch University https://scholar.sun.ac.za

ABBREVIATIONS

BSFL	Black soldier fly larva
CI	Confidence interval
DF	Degrees of freedom
DHA	Docohexaenoic acid
DPA	Docosapentaenoic acid
EPA	Eicosapentaenoic acid
FA	Fatty acid
MUFA	Monounsaturated fatty acid
PICOS	Population, intervention, comparator, outcome, study design
PRISMA	Preferred reporting items for systematic reviews and analyses
PUFA	Polyunsaturated fatty acid
SD	Standard deviation
SE	Standard error
SFA	Saturated fatty acid
UFA	Unsaturated fatty acid

Stellenbosch University https://scholar.sun.ac.za

TABLE OF CONTENTS

DECLARATION i

ABSTRACT ii

OPSOMMING iii

DEDICATION iv

ACKNOWLEDGEMENTS v

ABBREVIATIONS vii

CHAPTER 1 1

General introduction 1

1.1 References 3

CHAPTER 2 5

Methodology 5

2.1 Systematic review steps 5

2.2 Step 1 - Formulation of the review question 6

2.2.1 Population 6

2.2.2 Intervention 7

2.2.3 Comparator 7

2.2.4 Outcome 8

2.2.5 Study design 8

2.3 Step 2 - Determination of the eligibility criteria 9

2.4 Step 3 - Literature search 10

2.5 Step 4 - Study selection 11

2.5.1 Identification 12

2.5.2 Screening 12

2.5.3 Eligibility 12

2.5.4 Inclusion 12

2.6 Step 5 - Data collection 14

Stellenbosch University https://scholar.sun.ac.za

2.7 Step 6 - Risk of bias assessment 17
2.8 Step 7 - Meta-analysis 17
2.9 Step 8 - Review synthesis 21
2.10 References 21
CHAPTER 3 24
Qualitative data review 24
3.1 Publication details 24
3.1.1 Year of publication 24
3.1.2 Publication journal 25
3.1.3 Affiliated country 26
3.2 Methodology 27
3.2.1 Research focus 27
3.2.2 Study design and rearing setting 28
3.2.3 Total study duration 29
3.2.4 Pre-harvest purging and killing method 30
3.3 Participants: Black soldier fly larvae 31
3.3.1 Total number of black soldier fly larvae 31
3.3.2 Life stage at harvest 32
3.3.3 Age of black soldier fly larvae at harvest 32
3.4 Interventions 33
3.4.1 Number of intervention groups 33
3.4.2 Number of substrates 34
3.4.3 Inclusion of manure 34
3.5 Results 35
3.5.1 Rearing substrate formulation 35
3.5.2 Rearing substrate proximate composition 36
3.5.3 Rearing substrate fatty acid profile 36
3.5.4 Black soldier fly larvae proximate composition 37
3.5.5 Black soldier fly larvae fatty acid profile 38

Stellenbosch University https://scholar.sun.ac.za

3.6 Inclusion in meta-analyses 38
3.7 References 39
CHAPTER 4 47
Meta-analyses of saturated fatty acids 47
4.1 Summary statistics 48
4.2 Capric acid 51
4.3 Lauric acid 52
4.4 Myristic acid 53
4.5 Palmitic acid 54
4.6 Margaric acid 55
4.7 Stearic acid 56
4.8 Arachidic acid 57
4.9 Behenic acid 58
4.10 Total saturated fatty acids 59
4.11 Discussion 60
4.12 Reference 61
CHAPTER 5 65
Meta-analyses of monounsaturated fatty acids 65
5.1 Summary statistics 66
5.2 Myristoleic acid 69
5.3 Pentadecenoic acid 70
5.4 Palmitoleic acid 70
5.5 Heptadecenoic acid 71
5.6 Oleic acid 72
5.7 Vaccenic acid 73
5.8 Gondoic acid 74
5.9 Total monounsaturated fatty acids 75
5.10 Discussion 76
5.11 References 77

Stellenbosch University https://scholar.sun.ac.za

CHAPTER 6 81

Meta-analyses of polyunsaturated fatty acids 81

6.1 Summary statistics 82

6.2 Linoleic acid 85

6.3 α-Linolenic acid 87

6.4 γ-Linolenic acid 88

6.5 Stearidonic acid 89

6.6 Eicosadienoic acid 90

6.7 Arachidonic acid 91

6.8 Eicosapentaenoic acid 91

6.9 Docosapentaenoic acid 93

6.10 Docohexaenoic acid 93

6.11 Total omega-6 and omega-3 fatty acid concentration 94

6.11.1 Omega-6 fatty acids 95

6.11.2 Omega-3 fatty acids 96

6.12 Total polyunsaturated fatty acids 97

6.13 Discussion 98

6.14 References 100

CHAPTER 7 104

Discussion 104

7.1 Publications 104

7.2 Saturated fatty acids 108

7.3 Monounsaturated fatty acids 109

7.4 Polyunsaturated fatty acids 110

7.5 Conclusion 111

7.6 References 112

Stellenbosch University https://scholar.sun.ac.za

LIST OF TABLES

Table 2.1: Criteria used to determine inclusion of studies in the systematic review 9

Table 2.2: Criteria used to determine exclusion of studies in the systematic review 10

Table 2.3: Number of records identified during literature search of three databases..... 11

Table 2.4: Google form for qualitative data collection 14

Table 2.5: Example of quantitative data collection form 16

Table 4.1: List of saturated fatty acids identified as present in black soldier fly larval fatty acid profile 48

Table 4.2: Summary statistics regarding levels of individual saturated fatty acids (SFAs) in black soldier fly larval fatty acid profile (in % of total fatty acids)..... 49

Table 4.3: Average effect sizes (with confidence intervals) of the different fatty acids and total saturated fatty acid calculated by means of meta-analyses 61

Table 5.1: List of monounsaturated fatty acids identified as present in the black soldier fly larval fatty acid profile 66

Table 5.2: Summary statistics regarding concentrations of individual monounsaturated fatty acids (MUFAs) in black soldier fly larval fatty acid profile (in % of total fatty acids)..... 67

Table 5.3: Average effect size (with confidence intervals) of rearing substrate composition on the difference individual monounsaturated fatty acid concentrations calculated by the meta-analyses..... 77

Table 6.1: List of polyunsaturated fatty acids identified as present in the black soldier fly larval fatty acid profile 82

Table 6.2: Summary statistic regarding levels of individual polyunsaturated fatty acids (PUFAs) in black soldier fly larval fatty acid profile (in % of total fatty acids) 83

Table 6.3: Average effect size (with confidence intervals) of rearing substrate composition on the different individual polyunsaturated fatty acid concentrations calculated by the meta-analyses..... 99

Table 7.1: Summary of meta-analysis statistical results..... 106

Stellenbosch University https://scholar.sun.ac.za

LIST OF FIGURES

Figure 2.1: Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram. Adapted from Moher et al., (2019) 13

Figure 3.1: Year of publication of included studies 25

Figure 3.2: Journal of publication of included studies 26

Figure 3.3: Geographical distribution of included studies 27

Figure 3.4: Research focus of included studies 28

Figure 3.5: Study design of included studies 29

Figure 3.6: Rearing setting of included studies 29

Figure 3.7: Total study duration of included studies 30

Figure 3.8: Employment of pre-harvest purging in included studies 30

Figure 3.9: Killing method employed by included studies 31

Figure 3.10: Total number of black soldier fly larvae used in each study 31

Figure 3.11: Life stage of black soldier fly larvae at harvest 32

Figure 3.12: Age of black soldier fly larvae at harvest 33

Figure 3.13: Number of intervention groups in included studies 34

Figure 3.14: Number of substrates in included studies 34

Figure 3.15: Inclusion of manure in rearing substrates in included studies 35

Figure 3.16: Study reporting of rearing substrate formulation 36

Figure 3.17: Study reporting of rearing substrate proximate composition 36

Figure 3.18: Study reporting of rearing substrate fatty acid profile 37

Figure 3.19: Study reporting of black soldier fly larvae proximate composition 37

Figure 3.20: Study reporting of black soldier fly larvae fatty acid profile 38

Figure 3.21: Study inclusion in meta-analyses based on criteria 39

Figure 4.1: Software output of meta-analysis of capric acid concentration of black soldier fly larval fatty acid profile 52

Stellenbosch University https://scholar.sun.ac.za

Figure 4.2: Software output of meta-analysis of lauric acid concentration of black soldier fly larval fatty acid profile 53

Figure 4.3: Software output of meta-analysis of myristic acid concentration of black soldier fly larval fatty acid profile 54

Figure 4.4: Software output of meta-analysis of palmitic acid concentration of black soldier fly larval fatty acid profile 55

Figure 4.5: Software output of meta-analysis of margaric acid concentration of black soldier fly larval fatty acid profile 56

Figure 4.6: Software output of meta-analysis of stearic acid concentration of black soldier fly larval fatty acid profile 57

Figure 4.7: Software output of meta-analysis of arachidic acid concentration of black soldier fly larval fatty acid profile 58

Figure 4.8: Software output of meta-analysis of behenic acid concentration of black soldier fly larval fatty acid profile 59

Figure 4.9: Software output of meta-analysis of the total saturated fatty acid concentration of black soldier fly larval fatty acid profile 60

Figure 5.1: Software output of meta-analysis of myristoleic acid concentration of black soldier fly larval fatty acid profile 69

Figure 5.2: Software output of meta-analysis of pentadecenoic acid concentration of black soldier fly larval fatty acid profile 70

Figure 5.3: Software output of meta-analysis of palmitoleic acid concentration of black soldier fly larval fatty acid profile 71

Figure 5.4: Software output of meta-analysis of heptadecenoic acid concentration of black soldier fly larval fatty acid profile 72

Figure 5.5: Software output of meta-analysis of oleic acid concentration of black soldier fly larval fatty acid profile 73

Figure 5.6: Software output of meta-analysis of vaccenic acid concentration of black soldier fly larval fatty acid profile 74

Figure 5.7: Software output of meta-analysis of gondoic acid concentration of black soldier fly larval fatty acid profile 75

Figure 5.8: Software output of meta-analysis of the total concentration of monounsaturated fatty acids of black soldier fly larval fatty acid profile 76

Stellenbosch University https://scholar.sun.ac.za

Figure 6.1: Software output of meta-analysis of linoleic acid concentration of black soldier fly larval fatty acid profile 86

Figure 6.2: Software output of meta-analysis of α-linolenic acid concentration of black soldier fly larval fatty acid profile 88

Figure 6.3: Software output of meta-analysis of γ-linolenic acid concentration of black soldier fly larval fatty acid profile 89

Figure 6.4: Software output of meta-analysis of stearidonic acid concentration of black soldier fly larval fatty acid profile 90

Figure 6.5: Software output of meta-analysis of eicosadienoic acid concentration of black soldier fly larval fatty acid profile 90

Figure 6.6: Software output of meta-analysis of arachidonic acid concentration of black soldier fly larval fatty acid profile 91

Figure 6.7: Software output of meta-analysis of eicosapentaenoic acid concentration of black soldier fly larval fatty acid profile 92

Figure 6.8: Software output of meta-analysis of docosapentaenoic acid concentration of black soldier fly larval fatty acid profile 93

Figure 6.9: Software output of meta-analysis of docohexaenoic acid concentration of black soldier fly larval fatty acid profile 94

Figure 6.10: Software output of meta-analysis of total omega 6 fatty acids concentration of black soldier fly larval fatty acid profile 96

Figure 6.11: Software output of meta-analysis of total omega 3 fatty acids concentration of black soldier fly larval fatty acid profile 96

Figure 6.12: Software output of meta-analysis of the total polyunsaturated fatty acid concentration of black soldier fly larval fatty acid profile 98

Stellenbosch University https://scholar.sun.ac.za

LIST OF EQUATIONS

Equation 2.1: Standard deviation calculation 18

Equation 2.2: Study weight calculation..... 19

Equation 2.3: Average effect size calculation..... 19

Equation 2.4: Chi^2 test statistic calculation..... 19

Equation 2.5: Degrees of freedom calculation..... 19

Equation 2.6: Tau^2 statistic calculation..... 20

Equation 2.7: Scaling factor C calculation..... 20

Equation 2.8: I^2 statistic calculation..... 20

Equation 2.9: Freeman-Tukey double arcsine transformation 21

Equation 4.1: Standard deviation equation..... 50

Equation 4.2: Freeman-Tukey double arcsine transformation 50

Equation 5.1: Standard deviation equation 68

Equation 5.2: Freeman-Tukey double arcsine transformation 68

Equation 6.1: Standard deviation calculation 84

Equation 6.2: Freeman-Tukey double arcsine transformation 85

Stellenbosch University https://scholar.sun.ac.za

LIST OF APPENDIXES

APPENDIX A 115

Table A 1: List of included studies and indication of meta-analysis inclusion 115

Table A 2: List of studies included in meta-analyses 118

APPENDIX B 119

Figure B 1: Software output of second meta-analysis of arachidic acid concentration of black soldier fly larval fatty acid profile 119

Figure B 2: Software output of second meta-analysis of behenic acid concentration of black soldier fly larval fatty acid profile 119

Figure B 3: Software output of preliminary meta-analysis of eicosapentaenoic acid concentration of black soldier fly larval fatty acid profile 119

Figure B 4: Software output of preliminary meta-analysis of docohexaenoic acid concentration of black soldier fly larval fatty acid profile 119

Stellenbosch University https://scholar.sun.ac.za

CHAPTER 1
General introduction

In 2015 the global community committed to the goal of ending hunger, food insecurity and malnutrition by 2030 (FAO *et al.*, 2021). Since the agreement was made, food security has steadily increased every year. Unfortunately, the COVID-19 pandemic led to a drastic decline in food security in 2020. In that year between 720 and 811 million people faced hunger, which was an increase of 118 million from the previous year (FAO *et al.*, 2021). The problem was especially prominent in developing regions such as Africa and Latin America. Outside of the pandemic, there are four major factors that negatively impact food security: conflict, climate variability, economic slowdowns and downturns and unaffordability of healthy food (FAO *et al.*, 2021).

The importance of concepts such as sustainable agriculture and circular economies are being increasingly recognised globally as the world tries to achieve these food security goals, while mitigating the negative effects of climate change, which are exacerbated by human activity.

The livestock production sector, along with other food production sectors, is charged with the challenge of producing sufficient amounts of affordable nutritious food in a sustainable manner. Besides the previously mentioned factors and a growing global population, this challenge is compounded by the increased scarcity of resources (Barragan-Fonseca *et al.*, 2017). The scarcity of these resources in turn leads to increased competition between human food and animal feed for valuable resources such as fish oil, soybean meal and grains (Van Huis, 2013).

Now more than ever, animal nutritionists are trying to formulate feeds that incorporate more sustainable raw materials. Insects have been identified as showing promise in this regard. Insects such as the black soldier fly (*Hermetia illucens*) can be reared on organic waste and by-products and have the ability to convert these low grade wastes into nutrient rich insect biomass (Surendra *et al.*, 2020). Black soldier fly larvae (BSFL) are rich in proteins and lipids and are characterised by a high feed conversion efficiency and growth rate (Van Huis, 2013). Thus far they have predominantly been utilized as a protein source, but their lipids show promise to be used as an energy supplement in animal feed (Spranghers *et al.*, 2017).

Stellenbosch University https://scholar.sun.ac.za

Lipids make up the second largest component of insects' composition after protein and is their main source of energy (Barragan-Fonseca *et al.*, 2017). Insects' lipid content and fatty acid profile are influenced by a number of factors, including species, life stage, nutrition and rearing conditions (St-Hilaire *et al.*, 2007; Liu *et al.*, 2017; Kawasaki *et al.*, 2019). Some of these factors, such as nutrition and rearing conditions, can be manipulated to some extent to change the fatty acid profile of the larvae to more closely meet the fatty acid requirements of their intended use.

Black soldier fly larvae are reared until either the final larval stage or the prepupal stage. Also known as the fifth and sixth instar, respectively. Numerous research has been published on the influence of the composition of the rearing substrate on the BSFL lipid content and fatty acid profile (Danieli *et al.*, 2019; Truzzi *et al.*, 2020; Schreven *et al.*, 2021). The general conclusion among publications has been that the fatty acid profile of the larvae is influenced by the rearing substrate composition, but there is less clarity as to which extent the concentrations of the individual fatty acids can be affected by nutritional changes.

The purpose of this study was to determine the extent of the effect of the rearing substrate composition on the BSFL fatty acid profile. A systematic review was undertaken with the objective to consolidate and compare primary research findings, thereby producing more accurate results and identifying gaps in the knowledge (Higgins *et al.*, 2019). Meta-analyses were performed as a part of the systematic review as a way to incorporate empirical data regarding the changes in concentration of individual fatty acids in the BSFL fatty acid profile as a response to changes in the rearing substrate composition. The meta-analyses were incorporated into this study as it is a way of statistically combining quantitative results, specifically the changes in the concentrations of individual fatty acids, from different studies and thereby providing more accurate results. The systematic review of published research would potentially elucidate the extent to which the topic has been researched and which specific research questions have not been addressed yet, thereby identifying research projects that should still be undertaken.

The consolidated research reported in this study could assist in the development of more dynamic BSFL rearing substrate formulation to facilitate optimal BSFL production for animal and human consumption. Thereby contributing to global goals of food security and a more sustainable agricultural sector.

Stellenbosch University https://scholar.sun.ac.za

1.1 References

Barragan-Fonseca, K.B., Dicke, M. & van Loon, J.J.A., 2017. Nutritional value of the black soldier fly (*Hermetia illucens* L.) and its suitability as animal feed - a review. J. Insects as Food Feed. 3, 105-120. https://doi.org/10.3920/JIFF2016.0055

Danieli, P.P., Lussiana, C., Gasco, L., Amici, A. & Ronchi, B., 2019. The effects of diet formulation on the yield, proximate composition, and fatty acid profile of the black soldier fly (*Hermetia illucens* L.) prepupae intended for animal feed. Animals. 9, 178 https://doi.org/10.3390/ani9040178

FAO, IFAD, UNICEF, WFP, & WHO., 2021. The state of food security and nutrition in the world 2021: transforming food systems for food security, improved nutrition and affordable healthy diets for all. FAO, Rome. https://doi.org/10.4060/cb4474en

Higgins, J.P.T., Thomas, J., Chandler, J., Cumpston, M., Li, T. & Welch, V.A. (editors), 2019. Cochrane handbook for systematic reviews of interventions. 2nd Edition. Ed: Higgins, J.P.T., Thomas, J., -Chandler, J., Cumpston, M., Li, T. and Welch, V.A., John Wiley & Sons, Chichester, England. Available from www.training.cochrane.org/handbook

Van Huis, A., 2013. Potential of insects as food and feed in assuring food security. Annu. Rev. Entomol. 58, 563-583. https://doi.org/10.1146/annurev-ento-120811-153704

Kawasaki, K., Hashimoto, Y., Hori, A., Kawasaki, T., Hirayasu, H., Iwase, S., Hashizume, A., Ido, A., Miura, C., Miura, T., Nakamura, S., Seyama, T., Matsumoto, Y., Kasai, K. & Fujitani, Y., 2019. Evaluation of black soldier fly (*Hermetia illucens*) larvae and pre-pupae raised on household organic waste, as potential ingredients for poultry feed. Animals. 9, 98. https://doi.org/10.3390/ani9030098

Liu, X., Chen, X., Wang, H., Yang, Q., ur Rehman, K., Li, W., Cai, M., Li, Q., Mazza, L., Zhang, J., Yu, Z. & Zheng, L., 2017. Dynamic changes of nutrient composition throughout the entire life cycle of black soldier fly. PLoS One. 12. https://doi.org/10.1371/journal.pone.0182601

Schreven, S.J.J., Yener, S., van Valenberg, H.J.F., Dicke, M. & van Loon, J.J.A., 2021. Life on a piece of cake: performance and fatty acid profiles of black soldier fly larvae fed oilseed by-products. J. Insects as Food Feed. 7, 35-49. https://doi.org/10.3920/JIFF2020.0004

Spranghers, T., Ottoboni, M., Klootwijk, C., Ovyn, A., Deboosere, S., De Meulenaer, B., Michiels, J., Eeckhout, M., De Clercq, P. & De Smet, S., 2017. Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates. J. Sci. Food Agric. 97, 2594-2600. https://doi.org/10.1002/jsfa.8081

Stellenbosch University https://scholar.sun.ac.za

St-Hilaire, S., Cranfill, K., McGuire, M.A., Mosley, E.E., Tomberlin, J.K., Newton, L., Sealey, W., Sheppard, C. & Irving, S., 2007. Fish offal recycling by black soldier fly produces a foodstuff high in omega-3 fatty acids. J. World Aquac. Soc. 38, 197-202. https://doi.org/10.1111/j.1749-7345.2007.00101.x

Surendra, K.C., Tomberlin, J.K., van Huis, A., Cammack, J.A., Heckmann, L.L. & Khanal, S.K., 2020. Rethinking organic wastes bioconversion: evaluating the potential of the black soldier fly (*Hermetia illucens* (L.)) (Diptera: Stratiomyidae) (BSF). Waste Manag. 117, 58-80. https://doi.org/10.1016/j.wasman.2020.07.050

Truzzi, C., Giorgini, E., Annibaldi, A., Antonucci, M., Illuminati, S., Scarponi, G., Riolo, P., Isidoro, N., Conti, C., Zarantoniello, M., Cipriani, R. & Olivotto, I. , 2020. Fatty acids profile of black soldier fly (*Hermetia illucens*): influence of feeding substrate based on coffee-waste silverskin enriched with microalgae. Anim. Feed Sci. Technol. 259, 114309. https://doi.org/10.1016/j.anifeedsci.2019.114309

Stellenbosch University https://scholar.sun.ac.za

CHAPTER 2
Methodology

A systematic review is a research approach whereby a researcher can collect and consolidate all the data from relevant literature in an attempt to answer a specific research question (Gough *et al.*, 2019, Lasserson *et al.*, 2019). It is therefore referred to as *secondary research*, where observational studies and studies involving randomised control trials are referred to as *primary research*. Eligibility criteria for the inclusion of studies in the review are prespecified to minimize selection bias and provide more reliable findings to assist in answering the research question. The systematic process is intended to allow for more objective findings and conclusions.

This study follows the systematic review protocol developed by the Cochrane Collaboration and described in the Cochrane Handbook for Systematic Reviews of Interventions (Higgins *et al.*, 2019c). The Cochrane Collaboration is an international not-for-profit organisation that undertakes and publishes systematic reviews focused on health care research (Green *et al.*, 2008). Their medically focused systematic reviews form an important part of *evidence based healthcare* (Sauerland & Seiler, 2005). The protocol developed for systematic reviews by the collaboration is also employed in other research fields (Weru *et al.*, 2021). The Cochrane Collaboration protocol was chosen for this review as it was found to be well documented and the handbook provided a step by step guide, including considerations and cautions that should be taken.

2.1 Systematic review steps

The systematic review protocol is broken down into a number of steps. The steps were performed in the required sequences, though some contiguous steps did overlap at times. Each step and the overlaps are discussed in detail in this chapter. The protocol steps are:

1. Formulation of the review question
2. Determination of the eligibility criteria
3. Literature search
4. Data collection
5. Risk of bias assessment
6. Meta-analysis

Stellenbosch University https://scholar.sun.ac.za

7. Review synthesis

2.2 Step 1 - Formulation of the review question

The aim of a systematic review is to address an answerable research question and fill gaps in scientific knowledge (Lasserson *et al.*, 2019). The review question functions as a guide for the conduction of successive steps and therefore the aim of the review should be incorporated into the question. The review question condenses the review aim and objectives into a single sentence. Systematic review questions are divided into two categories: broad scope and narrow scope questions. Both have advantages and disadvantages. A broad scope question could result in a comprehensive summary of evidence, but may require more resources to perform. A narrow scope question could result in a manageable amount of data, but the evidence may be inadequate to provide a meaningful answer (Thomas *et al.*, 2019). The construction of a comprehensive review question consists of several components, which are encapsulated by the acronym PICOS. The acronym stands for **P**opulation, **I**ntervention, **C**omparator, **O**utcome and **S**tudy design (Thomas *et al,*. 2019). In order to formulate the review question, each of these components were defined in the context of this study:

2.2.1 Population

The *population* is a comprehensive description of the group of study subjects that the review will focus on (McKenzie *et al.*, 2019). It should be defined in terms of important characteristics such as species, age, sex, etc. The definition of the population should however be in a broad enough sense to consider possible diversity of available studies, while simultaneously being narrow enough to ensure that the review question is answerable (McKenzie *et al.*, 2019). For this study the population was defined as:

- “Black soldier fly larvae (BSFL)/*Hermetia illucens* harvested at the fifth and/or sixth instar”

The species “black soldier fly larvae/*Hermetia illucens*” was specified as this was the only insect species of interest in this review. The fifth and sixth instar, also known as the last larval and pre-pupal stages, respectively, were chosen as they are the last developmental stages before the larvae stop feeding and pupate. They are also the most common

Stellenbosch University https://scholar.sun.ac.za

harvesting stages used for both research and industrial purposes and therefore the most relevant developmental stages for this review.

2.2.2 Intervention

The *intervention* refers to the experimental treatments that were tested by the publications that are considered for inclusion in the review (McKenzie *et al.*, 2019). Interventions are intrinsically complex and therefore the review question should define the intervention of interest broadly to be able to include variations of the intervention (McKenzie *et al.*, 2019). The variations of the intervention are inspected during a later step of the review process and judgments on their inclusion and relevance are made then. For this study the intervention was defined as:

- "Formulated rearing substrates or specifically selected substrate ingredients fed to the black soldier fly larvae"

The rearing substrates refer to any BSFL feeds that were formulated with the intention of altering the nutritional composition of the BSFL. The BSFL lipid metabolism functions similarly to that of non-ruminants in terms of the bioaccumulation and biosynthesis of fatty acids (Pastor *et al.*, 2015). Their fatty acid profile is consequently influenced by the composition of the rearing substrate. The intervention was therefore defined as stated above to specify that the review would investigate evidence of changes in the BSFL fatty acid profile due to changes in the rearing substrate composition.

2.2.3 Comparator

The *comparator* is an extension of the intervention as it refers to the specific comparisons made between the treatment groups (McKenzie *et al.*, 2019). The comparator describes the type of measurements made to determine the intervention effectiveness. For this study the comparator was defined as:

- "The fatty acid profile of the harvested black soldier fly larvae (BSFL)"

The defined comparator refers to the concentrations of individual fatty acids in the context of the total amount of fatty acids (% of total fatty acids) detected in the BSFL after they were reared on substrates with different compositions. In recent times animal nutrition research

Stellenbosch University https://scholar.sun.ac.za

has shifted its focus from simply trying to increase or decrease feed/food lipid content to manufacturing feed/food with specific fatty acid profiles that are tailored to the fatty acid requirements of the consumer, be that production-animals or humans. As previously stated, the BSFL lipid metabolism pathways are similar to those of non-ruminant animals and therefore influenced by the rearing substrate composition. This comparator would therefore allow for the collection of empirical data regarding the influence of rearing substrate composition of the fatty acid profile of the BSFL.

2.2.4 Outcome

The *outcome* refers to the specific differences in intervention measurements between intervention groups (McKenzie *et al.*, 2019). An outcome is chosen with the purpose of identifying applicable data that could contribute to the review objectives. It functions as a complement to the defined intervention and comparator. For this study the outcome was defined as:

- “Any changes in the concentrations of individual fatty acids in the BSFL due to changes in the rearing substrate composition”

The outcome was defined as stated above to specify the inclusion of studies that identified any changes in the concentration of individual fatty acids in the BSFL. The limitation of changes specifically due to rearing substrate composition was indicated to allow for the collection of empirical data that are sufficiently related for comparisons of interest.

2.2.5 Study design

The *study design* refers to the type of studies in terms of the experimental design that would be considered for inclusion in the systematic review (McKenzie *et al.*, 2019). Some study designs are more likely to provide relevant and reliable data than others in terms of a specific research question. For this study the study design was defined as:

- “Only randomised control trials”

Stellenbosch University https://scholar.sun.ac.za

This specification was made to allow the consolidation of data from different studies and the comparison of the effects of different interventions on the outcomes of different treatment groups. Other study designs, such as observational studies and literature reviews, do not necessarily allow for these comparisons.

After each component was defined, the review question was composed as follows:

“A systematic review to assess the effect of rearing substrate composition on the fatty acid profile of black soldier fly larvae (*Hermetia illucens*) when harvested at either the fifth or sixth instar.”

2.3 Step 2 - Determination of the eligibility criteria

Eligibility criteria are study characteristics that are used to determine if studies would be included in a systematic review (Lefebvre *et al.*, 2019). The eligibility criteria are specified before searching for and selecting studies for inclusion. This aspect distinguishes systematic reviews from literature reviews found in primary research publications (McKenzie *et al.*, 2019). Therefore, the eligibility criteria for this review were determined before the literature search was performed. The criteria were divided into two categories: inclusion criteria and exclusion criteria.

The inclusion criteria refer to a list of defined criteria that was used to form the basis of the key search phrases during the literature search step (Table 2.1). The criteria were kept to a minimum to reduce possible biases during the literature search that could arise from narrowing the scope of the search too much, possibly missing studies that could contribute to the review.

Table 2.1: Criteria used to determine inclusion of studies in this systematic review

Inclusion criteria
Studies focused of use of insects in the field of nutrition, waste management or biofuel synthesis
Studies that include black soldier fly larvae
Studies that include analyses of black soldier fly larvae chemical characteristics

Stellenbosch University https://scholar.sun.ac.za

The exclusion criteria comprised of a list of criteria that was used to exclude studies during the selection step of the review (Table 2.2). The criteria were used to determine each identified study's relevance to the scope of the review question and studies were subsequently excluded from the review if they were characterised by any of the criteria. For this study the list of exclusion criteria was more extensive than the list of inclusion criteria as it was used to reduce the number of identified studies to only include those that were deemed applicable to the review scope.

Table 2.2: Criteria used to determine exclusion of studies in this systematic review

Exclusion criteria
Aquaculture nutrition research without black soldier fly larvae rearing trials
Ruminant nutrition research without black soldier fly larvae rearing trials
Non-ruminant nutrition research without black soldier fly larvae rearing trials
Consumer survey research
Meat science focused research without black soldier fly larvae rearing trials
Insect nutrition research that excluded black soldier fly larvae
Microbiology research without nutritional composition analysis
Processing technology and methodology research
Immunology research

2.4 Step 3 - Literature search

Once the review question was defined and the eligibility criteria determined, the next step was to perform the literature search. This step consisted of the identification of relevant databases to use for the search, the selection of key search phrases that would provide the most comprehensive list of publications, and recording the number of publications identified through each database (Lefebvre *et al.*, 2019). An information specialist at the Stellenbosch University library, Elizabeth Will-Mollard, was consulted before the searches were performed.

Three databases were selected for the literature search: Scopus, Web of Science and CAB Abstracts. It was determined that these three databases would be sufficient to produce the least biased and most comprehensive record of relevant publications. Google Scholar was

Stellenbosch University https://scholar.sun.ac.za

excluded as a database as the search engine does not have a screening procedure or peer review process for publications and journals and therefore its inclusion might introduce a bias.

After the databases were selected, the key search phrases were defined. Only three key search phrases were chosen and no limitations or filters were used during the searches. Limitations and filters were omitted as they could introduce a bias. For this study the following key search phrases were selected based on the prespecified inclusion criteria: “black soldier fly”/ “*Hermetia illucens*” and “fatty acid”. The number of publications provided by each database was recorded and is presented in Table 2.3. Once the initial searches had been performed, a publication alert was set up that would send a notification of all new publications that were linked to the key search phrases. These publications were individually screened using the same methodology reported in the study selection step of this review.

Table 2.3: Number of records identified during literature search of three databases

Database	**Number of records**
Scopus	1037
Web of Science	779
CAB abstracts	122
Total	1938
Total after removal of duplicates	1518

2.5 Step 4 - Study selection

The literature search was followed by the study selection step. The study selection protocol implemented in this review was based on the Preferred Reporting Items for Systematic Reviews and Analyses (PRISMA) protocol as defined by Moher *et al.*, (2015). The protocol is simplified in the form of a flow diagram (Figure 2.1). The study selection step used the prespecified exclusion criteria to reduce the number of studies to only those that were relevant to the review. The study selection step was divided into four phases: *Identification*, *screening*, *eligibility* and *inclusion*. Each of the phases will be explained briefly.

Stellenbosch University https://scholar.sun.ac.za

2.5.1 Identification

The identification phase overlaps with the last part of the literature search step as it entails the identification of the publications acquired from the databases (Lefebvre *et al.*, 2019). The publication records were imported to the Mendeley referencing management software version 1.19.8 (2021). The records were compared using the software and duplicates were subsequently removed (Table 2.3).

2.5.2 Screening

The screening phase consisted of screening the identified publications using the prespecified exclusion criteria (Table 2.2). The publications were individually inspected and compared to the criteria and were then either noted as being viable for use in the review or excluded if they were characterized by any of the exclusion criteria (Lefebvre *et al.*, 2019). The screening was first performed only on the publication titles and studies were subsequently excluded. The screening was then repeated on the publication abstracts and again publications were excluded. The remaining number of publications were noted and used in the eligibility phase.

2.5.3 Eligibility

The eligibility phase is analogous to the screening phase as it involved comparing the full texts of the remaining publications to the exclusion criteria and determining their applicability to the review scope (Lefebvre *et al.*, 2019). Their outcomes and reported analyses were also examined in terms of their relevance to the review question. The eligibility phase concluded with the compilation of the list of publications that were selected to be included in the review.

2.5.4 Inclusion

The last phase of the study selection involved classifying the included publications into two categories. The first category comprised of the publications that would be included in the qualitative aspect (narrative) of the review and included all the studies that were deemed relevant after the eligibility phase. The second category was a subset of the first as it consisted of the publications that would be included in the quantitative aspect (meta-analysis) of the review. Each publication was assessed in terms of their analytical results to determine if they contained the analytical data required for the meta-analysis. The list of

Stellenbosch University https://scholar.sun.ac.za

included publications can be seen in Appendix A (Table A 1). Appendix A also includes an indication of the publications' inclusion in the meta-analyses.

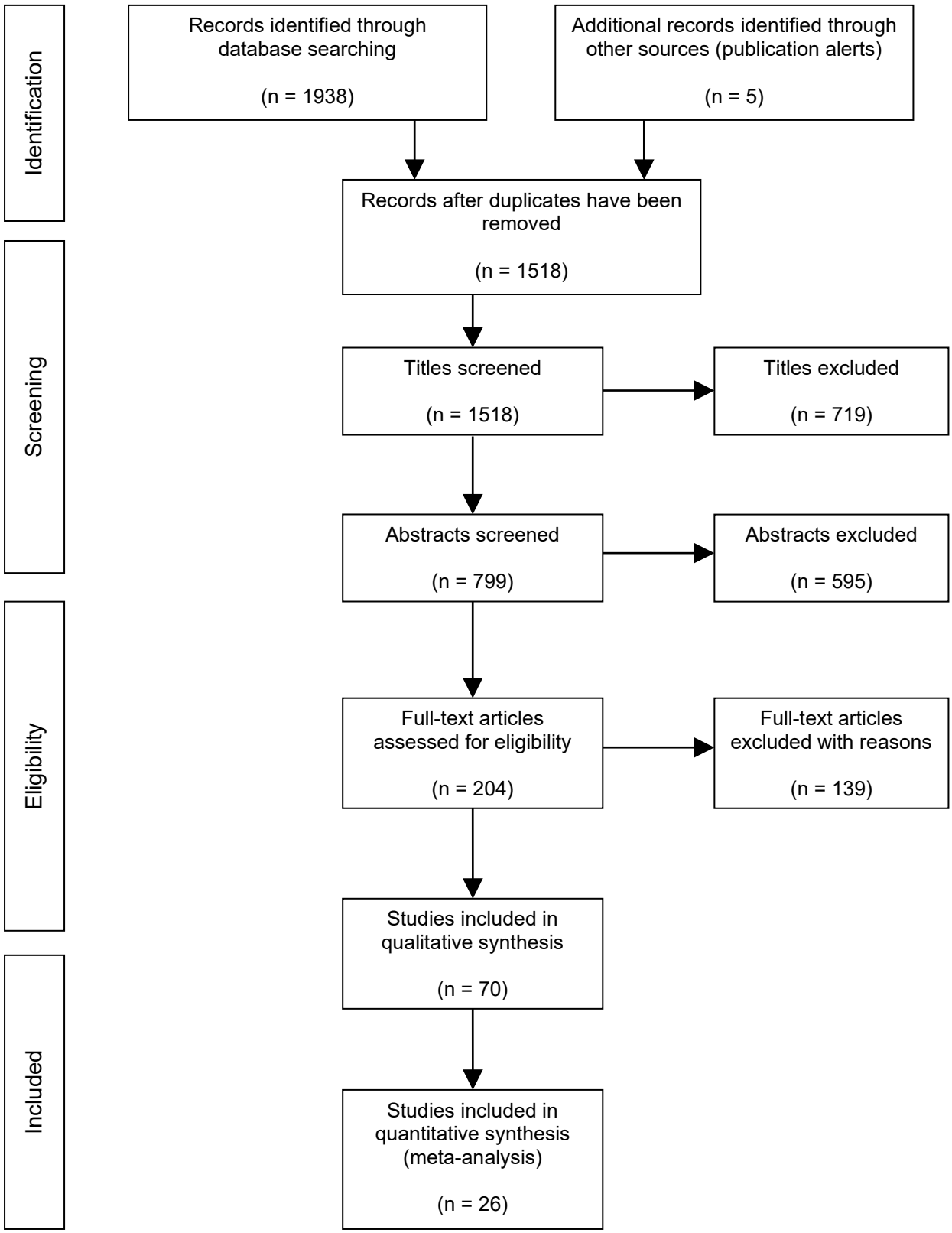


Figure 2.1: Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram. Adapted from Moher *et al.*, (2019)

Stellenbosch University https://scholar.sun.ac.za

2.6 Step 5 - Data collection

This step involved the collection of all data from the set of included studies regarding methodology, study subjects, setting, context, interventions, outcomes, results, publication and authors (Li *et al.*, 2019). In order to do this, two Google forms were developed to ensure that all relevant data would be collected. The first form was used to collect qualitative data, which were data regarding publication details and the narrative of the studies. The form was divided into different sections, each with a list of questions regarding that section's details. The form is illustrated by Table 2.4.

Table 2.4: Google form for qualitative data collection

Publication details	
Questions	**Options (if applicable)**
Record ID	
Publication title	
Authors	
Contact details	
Publication year	
Publication journal	
Country of first author affiliation	
Confirmation of inclusion in systematic review	- Yes / No / Unclear
Comments regarding publication details	
Methodology	
Research focus	- Feed and/or food - Waste management
Study design	- BSFL rearing trial - BSFL samples bought and analysed - Literature review
Randomised control trial	- Yes / No / Unclear
Total study duration	

Stellenbosch University https://scholar.sun.ac.za

Table 2.4: Continued

Methodology	
Rearing environment	- Laboratory - Mass rearing environment - Other
BSFL purged before harvest	- Yes / No / Unclear
Killing method	- Heat / Cold / Unclear
Comments regarding methodology	
Participants – black soldier fly larvae	
Total number of BSFL	
Life stage at harvest	- Larval stage - Pre-pupal stage - Both larval and pre-pupal stage - Unclear
Age at harvest (in days)	
Comments regarding BSFL	
Interventions	
Number of intervention groups	
Specific regarding intervention group treatments	
Length of intervention (doses and timing)	
Number of substrates	
Number of BSFL in each intervention group	
Inclusion of manure in interventions	- Yes / No / Unclear
Comments regarding interventions	
Outcomes	
Publication outcomes and possible time points collected	
Definition of outcomes	
Unit of measurement	
Outcomes correspond with systematic review outcomes	- Yes / No / Unclear
Comments regarding outcomes	

Stellenbosch University https://scholar.sun.ac.za

Table 2.4: Continued

Results		
Number of BSF analysed in each intervention group		
Total sample size		
Details regarding summary data		
Substrate formulation included	-	Yes / No / Unclear
Substrate proximate composition included	-	Yes / No / Unclear
Substrate fatty acid profile included	-	Yes / No / Unclear
BSF proximate composition included	-	Yes / No / Unclear
BSF fatty acid profile included	-	Yes / No / Unclear
Comments regarding results		
Discussion and conclusion		
Effect discussed		
Conclusion of publication		
Inclusion in meta-analyses	-	Yes / No / Unclear
Comments regarding discussion and conclusion		

The second data collection form was designed for the collection of quantitative data, specifically the BSFL fatty acid profile reported by the studies. An example of the data collection format is shown in Table 2.. These data were collected for meta-analysis purposes. The format in which the publication results were reported (mean ± SD or mean ± SE), the measurement unit and the analysis sample sizes were recorded (Higgins *et al.*, 2019a).

Table 2.5: Example of quantitative data collection form

Treatment group	Treatment group 1			
Fatty acid	mean	SD	SE	measurement unit
Lauric acid (C12:0)				
Myristic acid (C14:0)				
Palmitic acid (C16:0)				

Stellenbosch University https://scholar.sun.ac.za

2.7 Step 6 - Risk of bias assessment

The risk of bias assessment is also referred to as the *assessment of methodological quality*. It is used to determine the degree of bias in a publication that could lead to bias in the systematic review. Biases are divided into five categories: selection bias, performance bias, attrition bias, detection bias and reporting bias (Higgins *et al.*, 2019b). These categories and the strategies developed to determine the risk of bias were developed specifically for systematic reviews of medical and health care studies. It was developed due to the high risk of bias in these studies because of the amount of people involved in the conduction of the studies, be that medical professionals, patients or intervention administrators. Another reason for the development is the practical limitations in health care studies such as only being able to conduct observational studies as opposed to randomised control trials, which are intrinsically more biased (Boutron *et al.*, 2019). It is also due to the importance of accuracy in health care systematic reviews as their findings are implemented in evidence-based health care and could potentially influence a patient's medical treatment and health.

Animal nutrition and agricultural science studies are less prone to these kinds of biases. Firstly, research studies are designed beforehand specifically to minimize bias by incorporating randomization in trial designs. There is also transparency afforded to the research through the reporting of methodology, which further reduces bias.

The quantitative data collected for this review consisted of chemical analysis results that is less prone to biases than other forms of data. Therefore, it was deemed unnecessary to perform a risk of bias assessment of the included publications.

2.8 Step 7 - Meta-analysis

The quantitative data, specifically the black soldier fly larval (BSFL) fatty acid profile data, was collected during the data collection step for the purpose of performing meta-analyses. The concentrations of each detected fatty acid and the treatment group details were collected from each publication. Thereafter all data pertaining to an individual fatty acid were consolidated in subgroup datasets. This was done to allow for a meta-analysis to be performed for each fatty acid.

Stellenbosch University https://scholar.sun.ac.za

The systematic review software Review Manager version 5.4.1 (2020) was used to perform random-effects meta-analyses. The random-effects meta-analysis incorporates the assumption that the different studies estimated different, although related, intervention effects (Deeks *et al.,* 2019). While a fixed-effects meta-analysis assumes that there is one true effect, a random-effects meta-analysis assumes that there is a distribution of true effect sizes. The combined effect (average effect size) therefore represents the mean of the population of true effects. This allows the meta-analysis to address heterogeneity among the intervention effects.

Each meta-analysis consisted of a comparison of the fatty acid (FA) concentration differences reported by each publication. The input data for each publication was the analysis sample size, the mean concentration value and its standard deviation (SD) of the treatment group that reported the lowest concentration and the treatment group that reported the highest concentration. This allowed for the calculation of the largest difference in concentration reported by each publication. In cases where a publication reported the standard error (SE) instead of the standard deviation, the SD was calculated by using Equation 2.1 (Deeks *et al.,* 2019), where SE represents the standard error reported for the mean concentration and n represents the analysis samples size for that treatment group.

Equation 2.1: Standard deviation calculation

$$SD = SE \times \sqrt{n}$$

For each publication the software calculated an intervention effect size, also referred to as the difference in means, reported as a difference in concentration (% of total FA) and a confidence interval. In order to calculate the average effect size, the intervention effects were first weighted by the inverse of its variance. The random-effects meta-analysis incorporates the within-study variance and the between study variance, which is known as Tau^2. The weights are assigned using Equation 2.2, where W_i is the weight given to study i and V_i represents the sum of the within-study variance for that study and the between study variance, Tau^2.

Stellenbosch University https://scholar.sun.ac.za

Equation 2.2: Study weight calculation

$$W_i = \frac{1}{V_i}$$

The weighted average effect size, $\bar{W}$, was then computed by using Equation 2.3 , where Y_i is the intervention effect and W_i is the weight assigned to that intervention effect. The average effect size was then reported with an accompanying confidence interval (Deeks *et al.,* 2019).

Equation 2.3: Average effect size calculation

$$\bar{W} = \frac{\sum Y_i W_i}{\sum W_i}$$

Once the average effect size was calculated. The statistical tests for the overall effect and the heterogeneity were performed. The heterogeneity statistics consisted of the Chi^2 test, the Tau^2 statistic and the I^2 statistic. The Chi^2 test was performed and is simplified by Equation 2.4, where T_i is the squared deviation of each study.

Equation 2.4: Chi^2 test statistic calculation

$$Chi^2 = \sum W_i T_i^2 - \frac{(\sum W_i T_i)^2}{\sum W_i}$$

The Chi^2 statistic, also referred to as Q, was then accompanied by its degrees of freedom (df) which was calculated using Equation 2.5.

Equation 2.5: Degrees of freedom calculation

$$df = Number\ of\ studies - 1$$

The null hypothesis for the Chi^2 test was that the intervention effects were homogeneous and the alternative hypothesis was that there was heterogeneity among the intervention effects. This gave an indication of whether it would be possible to accurately compare the intervention effects of the different studies.

Stellenbosch University https://scholar.sun.ac.za

As previously mentioned Tau^2 (τ^2) is the between-study variance, thus an indication of the level of heterogeneity, and was calculated using Equation 2.6.

Equation 2.6: Tau^2 statistic calculation

$$\tau^2 = \frac{Q - df}{C} \; if \; Q > df$$

Or

$$\tau^2 = 0 \;\; if \; Q < df$$

C is a scaling factor used to ensure that τ^2 is on the same scale as the within-study variance and is calculated using Equation 2.7.

Equation 2.7: Scaling factor C calculation

$$C = \sum W_i - \frac{\sum W_i^2}{\sum W_i}$$

The I^2 statistic described the percentage of variability among the intervention effects that was due to heterogeneity rather than chance alone. It was calculated using Equation 2.8,

Equation 2.8: I^2 statistic calculation

$$I^2 = \left(\frac{Q - df}{Q}\right) \times 100\%$$

A basic guide for the interpretation of the I^2 statistic is as follows:

- 0% to 40% might not be important
- 30% to 60% may represent moderate heterogeneity
- 50% to 90% may represent substantial heterogeneity
- 75% to 100% is considerable heterogeneity

Preliminary meta-analyses were performed, but it was found that the heterogeneity was too large to justify a meta-analysis and that it would have a major effect on the accuracy of the results. The data were therefore transformed using the *Freeman-Tukey double arcsine transformation* (Freeman & Tukey, 1950), which is a data transformation used to decrease

Stellenbosch University https://scholar.sun.ac.za

heterogeneity and increase the normality of a dataset distribution. The transformation can be seen in Equation 2.9, where y_i is the original data point and $\hat{y}_i$ is the transformed data point. The equation below is the original formula proposed in the article by Freeman and Tukey. The two arcsine values can however also be divided by two with the purpose of having the transformed values on the same scale as the arcsine-square-root transformation, which is a different transformation utilised for percentage data transformation. The meta-analyses as described above were then performed with the transformed values. Therefore, the results of the meta-analyses were also reported on the transformed scale.

Equation 2.9: Freeman-Tukey double arcsine transformation

$$\hat{y}_i = \arcsin\sqrt{y_i/(100+1)} + arcsin\sqrt{(y_i+1)/(100+1)}$$

2.9 Step 8 - Review synthesis

The qualitative data were consolidated and was used for the narrative aspect of this review. The narrative review is discussed in Chapter 3. The meta-analyses performed on the data regarding the individual fatty acids were divided into three groups: saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). This was done to allow for the interpretation of the effect of the rearing substrate composition on the concentration of a particular fatty acid in the context of its saturation.

2.10 References

Boutron, I., Page, M.J., Higgins, J.P.T., Altman, D.G., Lundh, A. & Hróbjartsson, A., 2019. Chapter 7: Considering bias and conflicts of interest among the included studies. In: Cochrane handbook for systematic reviews of interventions. 2nd Edition. Ed: Higgins, J.P.T., Thomas, J., Chandler, J., Cumpston, M., Li, T. and Welch, V.A., John Wiley & Sons, Chichester, England. pp.177-204.

Chaimani, A., Caldwell, D.M., Li, T., Higgins, J.P.T. & Salanti, G., 2019. Chapter 11: Undertaking network meta-analyses. In: Cochrane handbook for systematic reviews of interventions. 2nd Edition. Ed: Higgins, J.P.T., Thomas, J., Chandler, J., Cumpston, M., Li, T. and Welch, V.A., John Wiley & Sons, Chichester, England. pp.285-320.

Stellenbosch University https://scholar.sun.ac.za

Deeks, J.J., Higgins, J.P.T. & Altmand, D.G. (editors), 2019. Chapter 10: Analysing data and undertaking meta-analyses. In: Cochrane handbook for systematic reviews of interventions. 2nd Edition. Ed: Higgins, J.P.T., Thomas, J., Chandler, J., Cumpston, M., Li, T. and Welch, V.A., John Wiley & Sons, Chichester, England. pp.241-284.

Freeman, M.F. & Tukey, J.W. 1950. Transformations related to the angular and the square root. Ann. Mathematical Stat., 21, 607-611.

Gough, D., Oliver, S. & Thomas, J., 2019. Systematic reviews. In: Paul Atkinson, ed., SAGE Research Methods Foundations. Ed: Atkinson, P., Delamont, S., Cernat, A., Sakshaug, J.W. & Williams, R.A., SAGE Publications, Ltd., London, England. https://www.doi.org/10.4135/9781526421036857078

Higgins, J.P.T., Li, T. & Deeks, J.J. (editors), 2019a. Chapter 6: Choosing effect measures and computing estimates of effect. In: Cochrane handbook for systematic reviews of interventions. 2nd Edition. Ed: Higgins, J.P.T., Thomas, J., Chandler, J., Cumpston, M., Li, T. and Welch, V.A., John Wiley & Sons, Chichester, England. pp.143-176.

Higgins, J.P.T., Savović, J., Page, M.J., Elbersm, R.G. & Sterne, J.A.C., 2019b. Chapter 8: Assessing risk of bias in a randomized trial. In: Cochrane handbook for systematic reviews of interventions. 2nd Edition. Ed: Higgins, J.P.T., Thomas, J., Chandler, J., Cumpston, M., Li, T. and Welch, V.A., John Wiley & Sons, Chichester, England. pp.205-228.

Higgins, J.P.T., Thomas, J., Chandler, J., Cumpston, M., Li, T. & Welch, V.A. (editors), 2019c. Cochrane handbook for systematic reviews of interventions. 2nd Edition. Ed: Higgins, J.P.T., Thomas, J., Chandler, J., Cumpston, M., Li, T. and Welch, V.A., John Wiley & Sons, Chichester, England. Available from www.training.cochrane.org/handbook

Lasserson, T.J., Thomas, J. & Higgins, J.P.T., 2019. Chapter 1: Starting a review. In: Cochrane handbook for systematic reviews of interventions. 2nd Edition. Ed: Higgins, J.P.T., Thomas, J., Chandler, J., Cumpston, M., Li, T. and Welch, V.A., John Wiley & Sons, Chichester, England. pp.3-12.

Lefebvre, C., Glanville, J., Briscoe, S., Littlewood, A., Marshall C., Metzendorf, M.I., Noel-Storr, A., Rader, T., Shokraneh, F., Thomas, J. & Wieland, L.S., 2019. Chapter 4: Searching for and selecting studies. In: Cochrane handbook for systematic reviews of interventions. 2nd Edition. Ed: Higgins, J.P.T., Thomas, J., Chandler, J., Cumpston, M., Li, T. and Welch, V.A., John Wiley & Sons, Chichester, England. pp.67-108.

Li, T., Higgins, J.P.T. & Deeks, J.J. (editors), 2019. Chapter 5: Collecting data. In: Cochrane handbook for systematic reviews of interventions. 2nd Edition. Ed: Higgins, J.P.T., Thomas, J., Chandler, J., Cumpston, M., Li, T. and Welch, V.A., John Wiley & Sons, Chichester, England. pp.109-142.

Stellenbosch University https://scholar.sun.ac.za

McKenzie, J.E., Brennan, S.E., Ryan, R.E., Thomas, H.J., Johnstron, R.V. & Thomas, J., 2019. Chapter 3: Defining the criteria for including studies and how they will be grouped for the synthesis. In: Cochrane handbook for systematic reviews of interventions. 2nd Edition. Ed: Higgins, J.P.T., Thomas, J., Chandler, J., Cumpston, M., Li, T. and Welch, V.A., John Wiley & Sons, Chichester, England. pp.33-66.

Moher, D., Shamseer, L., Clarke, M., Ghersi, D., Alessandro, L., Petticrew, M., Shekelle, P., Stewart, L.A. & PRISMA-P Group, 2015. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst. Rev. 4, 1-9. https://doi.org/10.1186/2046-4053-4-1

Sauerland, S. & Seiler, C.M., 2005. Role of systematic reviews and meta-analysis in evidence-based medicine. World J. Surg. 29, 582-287. https://doi.org/10.1007/ s00268-005-7917-7

Thomas, J., Kneale, D., McKenzie, J.E., Brennan, S.E. & Bhaumik, S., 2019. Chapter 2: Determining the scope of the review and the questions it will address. In: Cochrane handbook for systematic reviews of interventions. 2nd Edition. Ed: Higgins, J.P.T., Thomas, J., Chandler, J., Cumpston, M., Li, T. and Welch, V.A., John Wiley & Sons, Chichester, England. pp. 13-32.

Weru, J., Chege, P. & Kinyuru, J., 2021. Nutritional potential of edible insects: a systematic review of published data. Int. J. Trop. Insect Sci. 41, 2015-2037. https://doi.org/10.1007/s42690-021-00464-0

Stellenbosch University https://scholar.sun.ac.za

CHAPTER 3
Qualitative data review

The qualitative data collection step was the fifth step in the systematic review protocol and was performed after the study selection step was completed. The study selection step delivered 65 publications that were identified to be included in the systematic review (Table A 1). After the initial literature search and study selection had been completed, five more publications were identified following alerts from the databases. The qualitative data from the 70 publications were collected by means of a Google form that was divided into sections as described in the data collection methodology in Chapter 2 (Li *et al.*, 2019). The consolidated qualitative data (**Error! Reference source not found.**) are discussed in terms of the following sections: publication details, methodologies, the participants (black soldier fly larvae), interventions and results.

3.1 Publication details

3.1.1 Year of publication

Figure 3.1 shows the year of publication of the included studies. The earliest publication that was identified as relevant to this review through the literature search and study selection steps was published in 2007 (St-Hilaire *et al.*, 2007). No relevant publications were published between 2008 and 2014. Thereafter there was a steady increase in the number of publications starting from 2015 until 2021. The smaller number of publications identified for 2021 is misleading as this does not represent a full year of publications and this number is still expected to increase. From these publication dates it is evident that the importance of black soldier fly larvae's (BSFL) lipid content, fatty acid composition and the effect that nutrition has on it is being addressed in research.

Stellenbosch University https://scholar.sun.ac.za

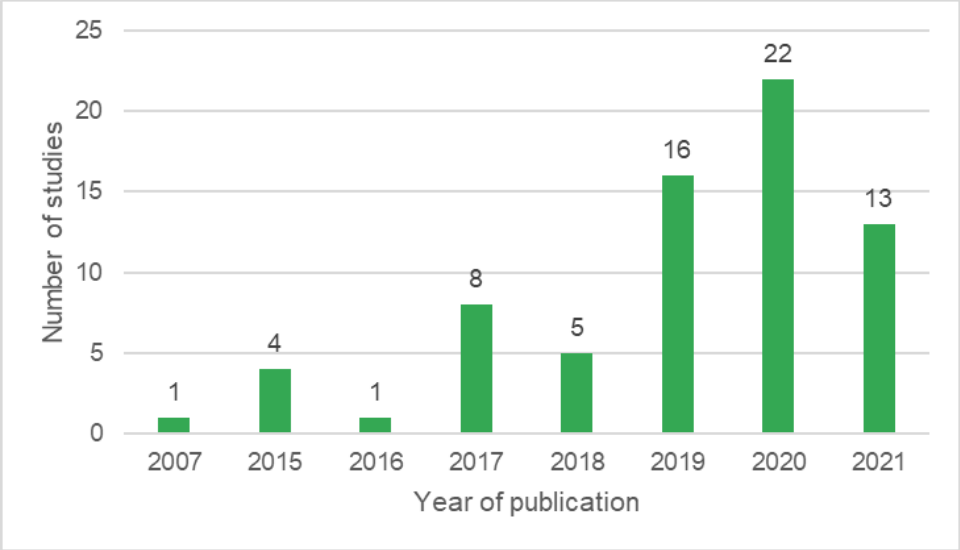


Figure 3.1: Year of publication of included studies

3.1.2 Publication journal

The included publications were published in a total of 34 journals (Figure 3.). The journals that published the most included studies were the “Journal of Insect Science” and “Journal of Insects as Food and Feed”, which published five articles each that were included in this review. It is interesting to note that the research fields of the various journals were broad and ranged from waste management and renewable energy to animal science and entomology. This indicated that the research regarding the BSFL fatty acids is a topic of interest for researchers from different fields and their importance in the aim of increasing the sustainability of various industries is being recognised.

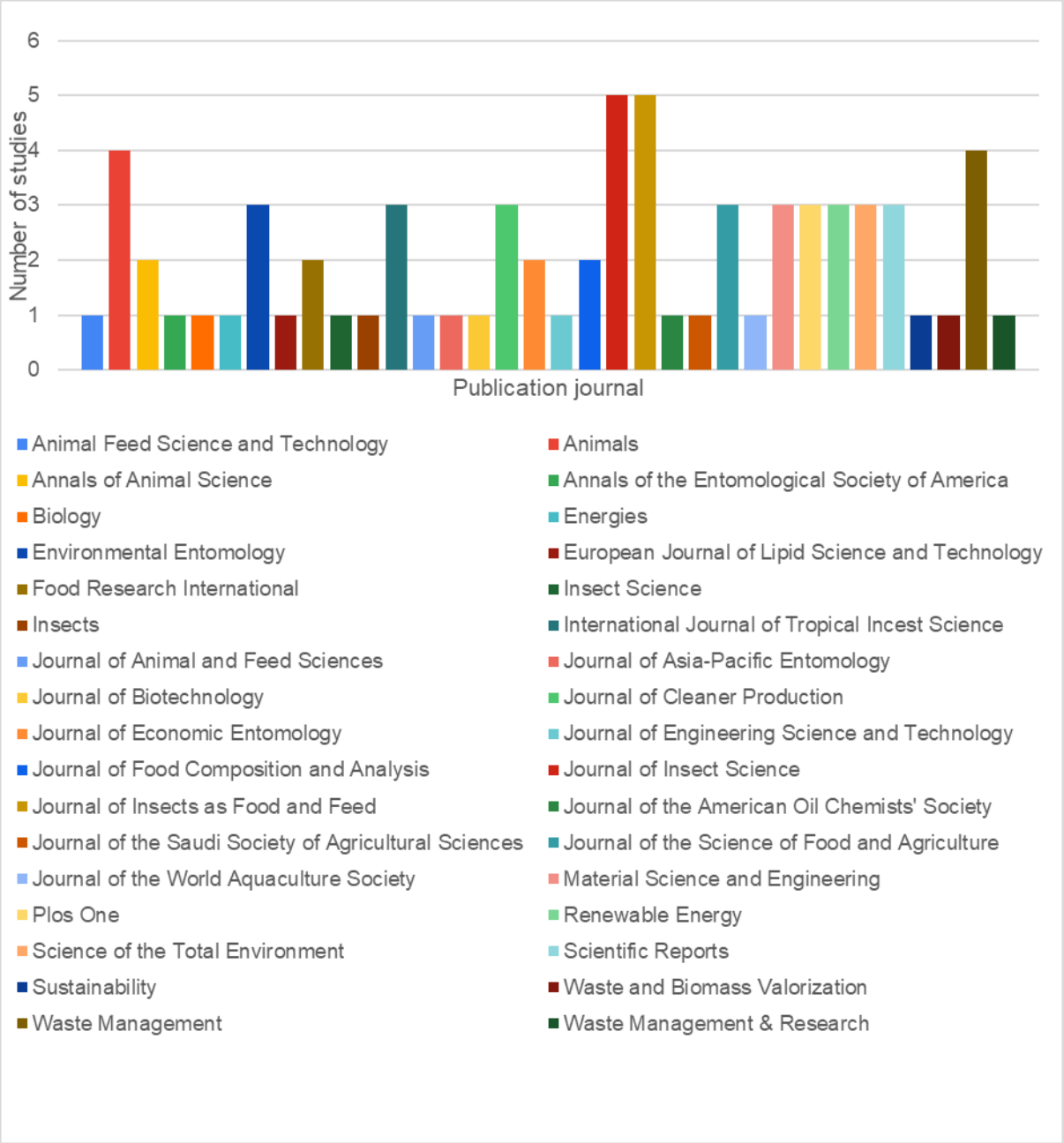


Figure 3.2: Journal of publication of included studies

3.1.3 Affiliated country

The first author's affiliated university details were used to determine the country of first author's affiliation. From Figure 3.3 it was seen that research relevant to this review was done in a total of 21 countries. The majority of the publications were from the northern hemisphere with Italy producing the most publications (14), followed by China and the United States of America, both with nine publications each. However, the geographic distribution is still broad with publications noted on most of the continents.

Stellenbosch University https://scholar.sun.ac.za

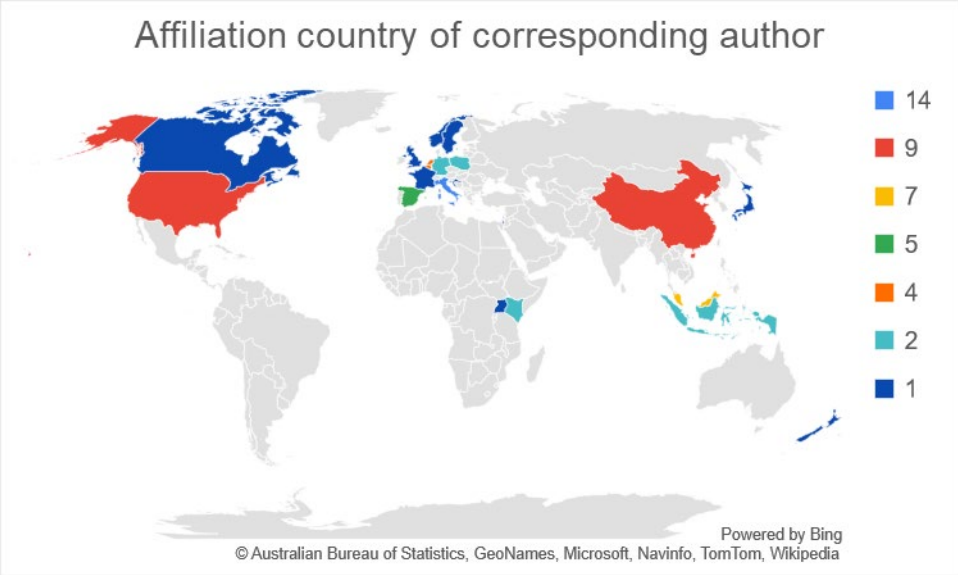


Figure 3.3: Geographical distribution of included studies

Based on the publication detail data, it was evident that there is a global increase in research interest surrounding the nutritional value of black soldier fly larvae, focusing on their fatty acid profile.

3.2 Methodology

3.2.1 Research focus

To collect data regarding the research-focus of the included studies, three categories were designated. These categories were “Feed and/or food”, which refers to black soldier fly larvae (BSFL) nutrition focused studies and “Waste management”, which refers to research that was focused on the management of specific waste or by-products through the use of BSFL. The third category was labelled “Both”, which refers to studies that had an equal focus on both of the aforementioned topics. The majority of the studies focused on both nutrition and waste management, as their aims were to evaluate the influence on the nutritional value of BSFL by including different waste or by-products in the rearing substrates (Figure 3.). This showed that research regarding BSFL is aimed at finding more sustainable ways to rearing larvae with increased nutritional value.

Stellenbosch University https://scholar.sun.ac.za

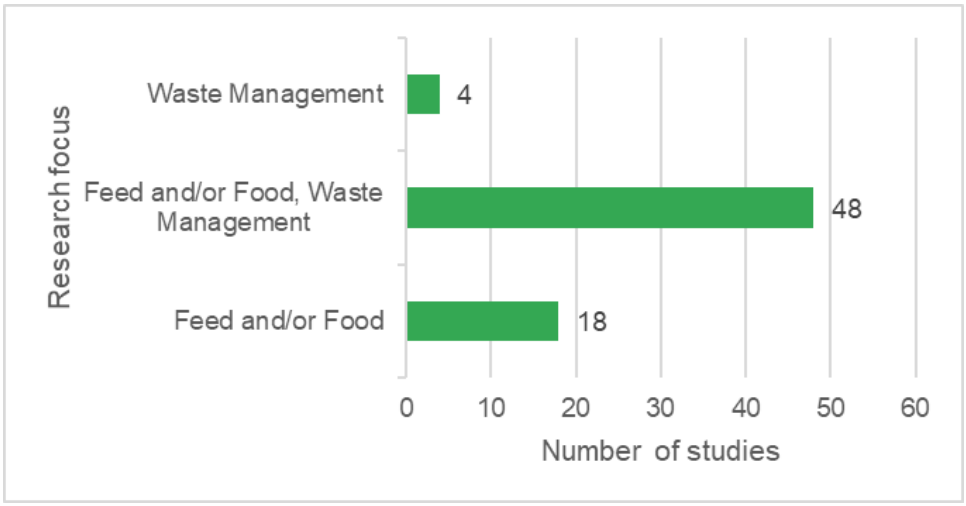


Figure 3.4: Research focus of included studies

3.2.2 Study design and rearing setting

The study design component of the review question indicated that only randomised control trials would be considered for this review. This was however not used as an exclusion criterion, but as an indicator as to which of the included studies would be eligible to be included in the meta-analysis aspect of this review. Therefore, the study design of the included studies was also recorded and the results are depicted in Figure 3.. Out of the 70 included studies, 59 employed randomised control trials. The remaining studies either bought BSFL samples from private rearing companies and analysed them (Caligiani *et al.*, 2018, 2019; Rabani *et al.*, 2019; Matthaeus *et al.*, 2019; Campbell *et al.*, 2020) or performed narrative reviews of the nutritional value of BSFL reported by other studies (Pastor *et al.*, 2015; Barragan-Fonseca *et al.*, 2017; Koutsos *et al.*, 2019; Benzertiha *et al.*, 2020; Surendra *et al.*, 2020; Weru *et al.*, 2021). It was decided that these studies would still be included in this review as it would add to the understanding of the current state of research based on both primary and secondary research. Out of the 59 studies that performed randomised control trials, 51 reared the BSFL in a laboratory setting and eight reared the BSFL in a mass rearing environment (Figure 3.). This was expected as this specific field of research is still relatively new in the sense that many studies were the first of their kind and therefore most of the studies were still being conducted on a smaller scale and many research institutions do not have the infrastructure to rear BSFL on a large scale for research purposes.

Stellenbosch University https://scholar.sun.ac.za

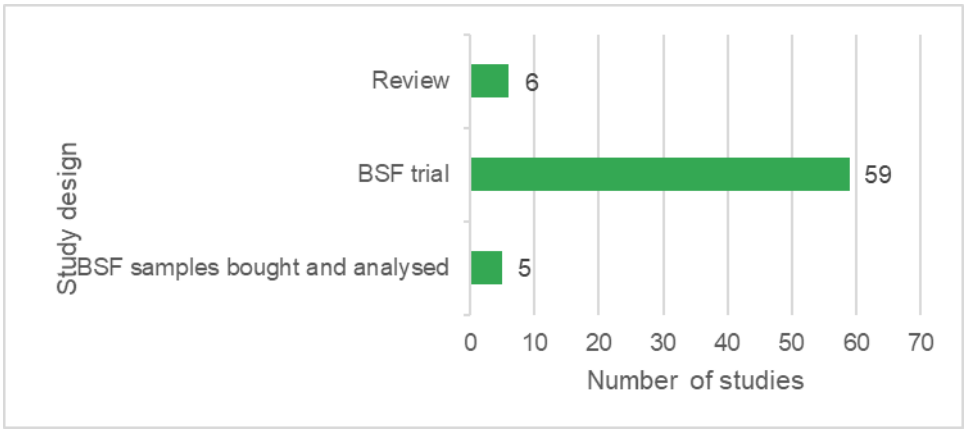


Figure 3.5: Study design of included studies

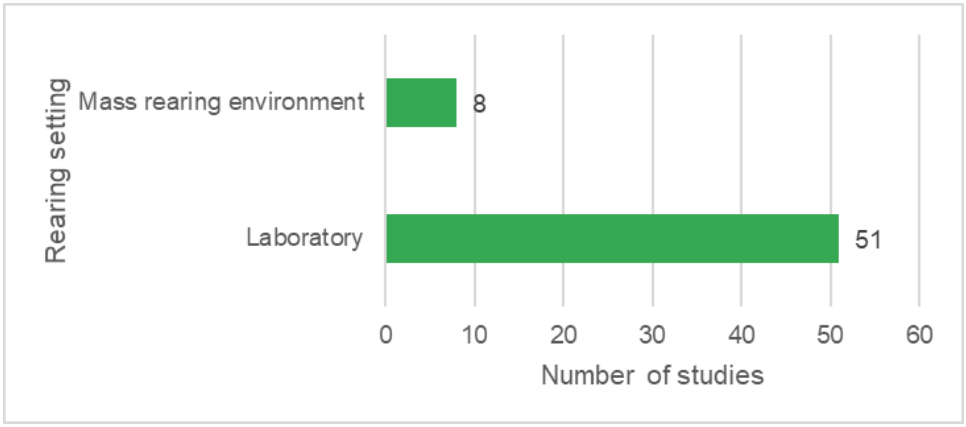


Figure 3.6: Rearing setting of included studies

3.2.3 Total study duration

Only 28 of the included studies reported the total study duration and from these the duration ranged from 8 days (Erbland *et al.*, 2020) up to 65 days (Barbi *et al.*, 2020). A large number of the included studies did not report the study durations in their methodologies (Figure 3.). Many of the studies ended their trials based on the life stage of the BSFL and not a specific number of days, which lead to the wide range of study durations. There was also variation on the different stages investigated in the trials as some of the studies investigated the whole life cycle of the BSFL or multiple stages, where the growth stages only accounted for a part of the study. This does however indicate that there is no standardised study design for BSFL growth trials in terms of durations, which could possibly be a source of heterogeneity among results.

Stellenbosch University https://scholar.sun.ac.za

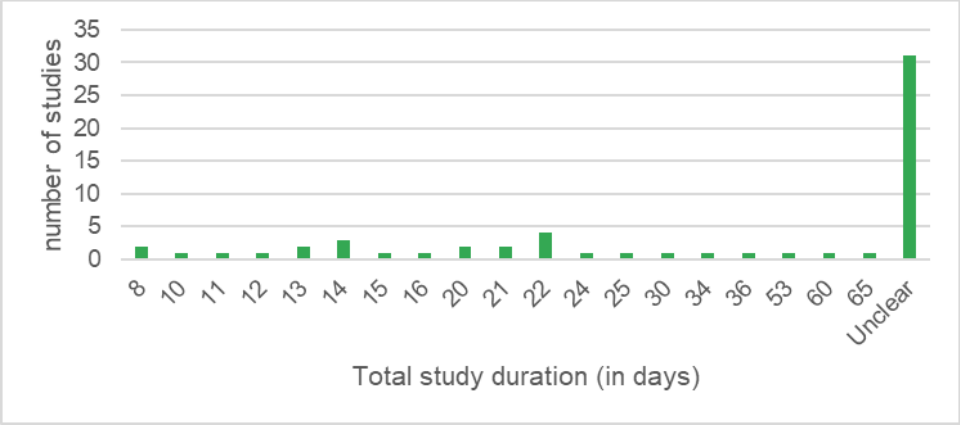


Figure 3.7: Total study duration of included studies

3.2.4 Pre-harvest purging and killing method

There was a great deal of variation in the methodologies in regard to pre-harvest purging (Figure 3.) and killing method (Figure 3.). Studies have suggested that purging and killing method could influence the BSFL fatty acid profile (Caligiani *et al.*, 2019; Egnew *et al.*, 2021). The differences in methodologies between studies would however not influence differences in fatty acid profiles reported within an individual study. Therefore, studies were not excluded based on these methodological choices. This does however again indicate that there is a lack of standardisation for studies that investigate the BSFL fatty acid profile.

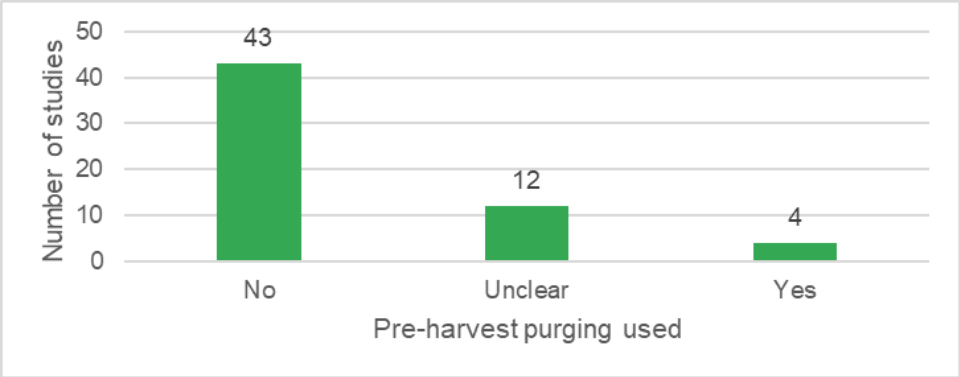


Figure 3.8: Employment of pre-harvest purging in included studies

Stellenbosch University https://scholar.sun.ac.za

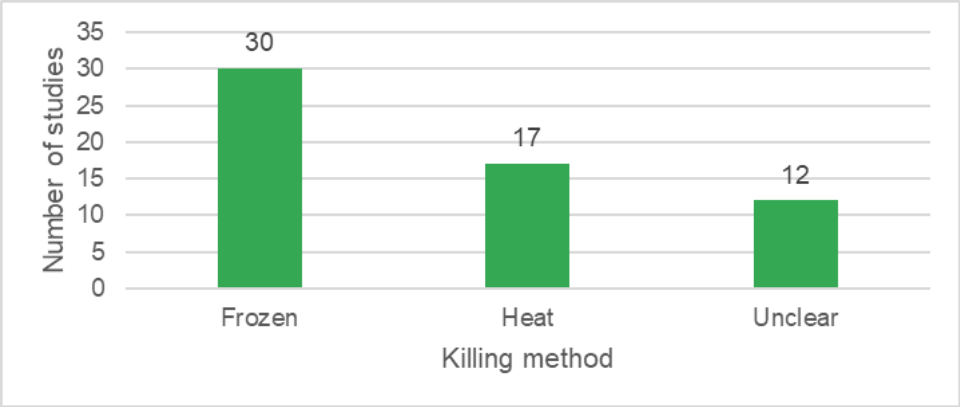


Figure 3.9: Killing method employed by included studies

3.3 Participants: Black soldier fly larvae

3.3.1 Total number of black soldier fly larvae

Data regarding the total number of black soldier fly larvae (BSFL) used in each study was recorded. It ranged from 20 larvae (Abduh *et al.*, 2018) to approximately 82500 larvae (Guil-Guerrero *et al.*, 2020) and is shown in Figure 3.. It should be noted that many of the studies did not report the total number of BSFL used in the trials. From the figure it can also be seen that most of the studies used a smaller number of BSFL, which corresponds with the larger number of studies conducted in a laboratory environment as they have a smaller capacity than large scale production units.

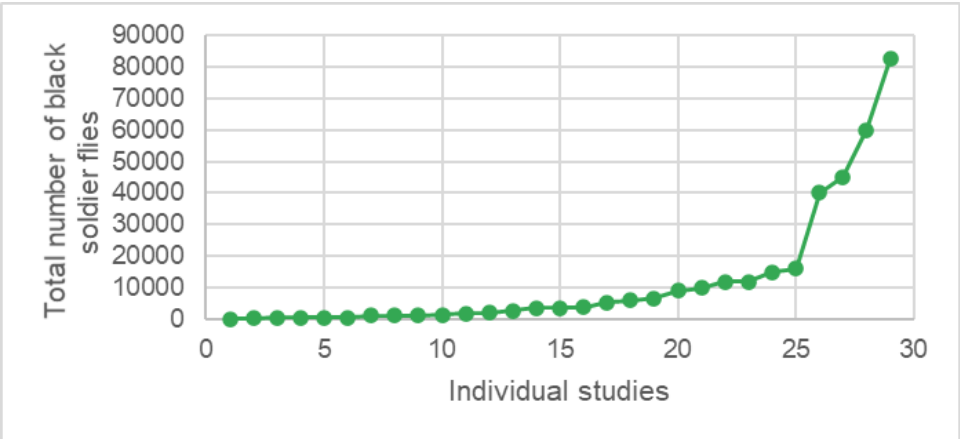


Figure 3.10: Total number of black soldier fly larvae used in each study

Stellenbosch University https://scholar.sun.ac.za

3.3.2 Life stage at harvest

The life stage at which the BSFL were harvested was noted as this is known to influence the fatty acid profile (Giannetto *et al.*, 2020) and is reported in Figure 3.2. Three categories were designated based on the harvesting life stage that is commonly used in both research and industry. These categories were “larval stage”, “pre-pupal stage” and “both”, which indicated that the studies harvested all the BSFL when a predetermined percentage had transitioned into the pre-pupal stage. The majority of the studies harvest the BSFL at the prepupal stage. This could be due to convenience as BSFL change colour from a cream colour in the last larval stage to a brown colour when they enter the pre-pupal stage, which makes this life stage easy to recognise. Black soldier fly larvae also self-harvest, which means that they stop feeding and leave the moist environment of the rearing substrate to find a dry environment to pupate. This movement is then an indication of the end of the prepupal stage. Generally the studies that focused more on waste management had a longer rearing time as the nutritional composition of the rearing substrate was designed for waste conversion and not necessarily for optimal nutrient accumulation (Grossule *et al.*, 2020). There has however been a trend in nutritional research towards a shorter rearing time and a younger harvesting age as this has the consequence of decreasing the quantity of resources needed for each rearing cycle.

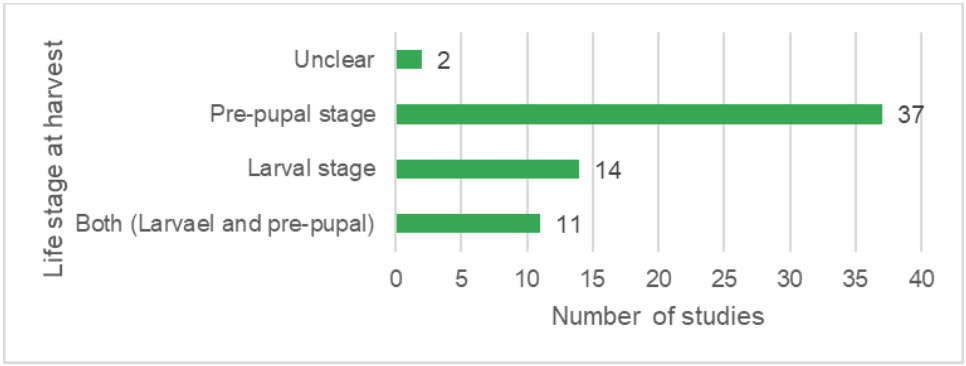


Figure 3.21: Life stage of black soldier fly larvae at harvest

3.3.3 Age of black soldier fly larvae at harvest

The age of the BSFL at harvest, in days, varied a great deal and ranged from 11 days until 60 days (Figure 3.3). Again, this had to do with the nutritional composition of the rearing substrates used in each of the studies and harvesting based on life stages and not on BSFL age in days. This can also be seen in the large number of studies that did not report the BSFL age at harvest.

Stellenbosch University https://scholar.sun.ac.za

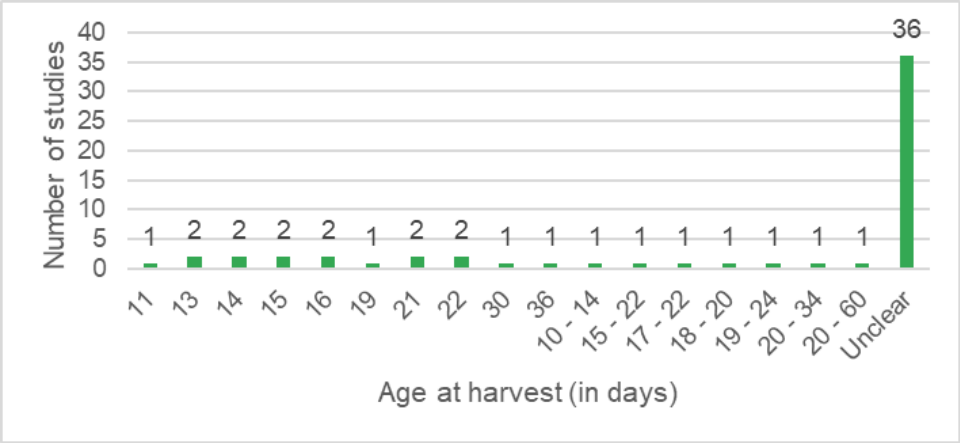


Figure 3.32: Age of black soldier fly larvae at harvest

3.4 Interventions

The data collection section “Interventions” refers to all the details pertaining to the different treatment groups. Through the study selection step, only studies which investigated the effect of nutrition on the BSFL fatty acid profile were included in the review. This section explicates the most prominent differences that were found between studies, particularly in the rearing substrate formulation and nutritional value.

3.4.1 Number of intervention groups

The number of intervention groups or treatment groups ranged from one to forty-nine (Figure 3.1). The studies that only had one treatment group were studies that investigated the difference between different insect species (Rabani *et al.*, 2019) and therefore only had one intervention group that consisted of BSFL. Others investigated methodological differences in chemical composition analysis (Caligiani *et al.*, 2018) or were limited by the amount of substrate available for the study (Abduh *et al.*, 2018). These studies were not included in the meta-analysis aspect of this review, but it was still deemed important to include them in the qualitative data collection. The largest number of intervention groups was 18, except for one outlier study that had 49 intervention groups. This was a very large study that aimed to develop a nutritional year plan by incorporating different seasonal organic wastes in the rearing substrate (Barbi *et al.*, 2020).

Stellenbosch University https://scholar.sun.ac.za

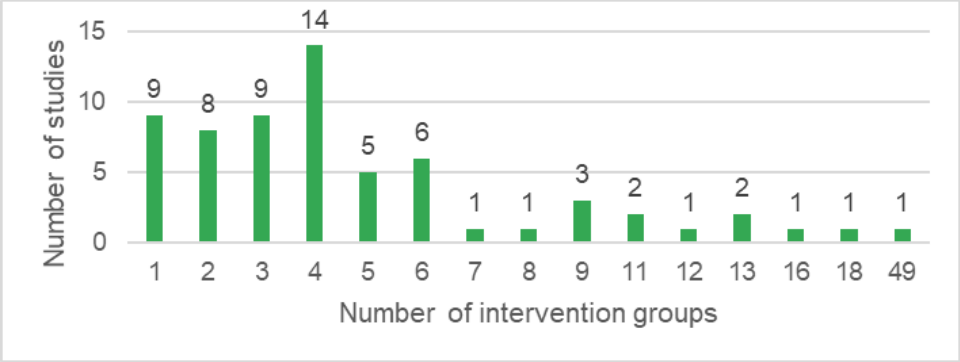


Figure 3.13: Number of intervention groups in included studies

3.4.2 Number of substrates

The number of substrates used in each study did not always correspond exactly with the number of intervention groups (Figure 3.). Even though all of the included studies investigated the effect of nutrition on the nutritional composition of the BSFL, some of the studies investigated the effect of feeding a treatment substrate for a specific time before harvest, in other words feeding strategy (Barroso *et al.*, 2019). Others investigated the effect of chemical analysis methodological differences when only using one formulated substrate (Caligiani *et al.*, 2018). Therefore, the number of substrates was also collected as qualitative data.

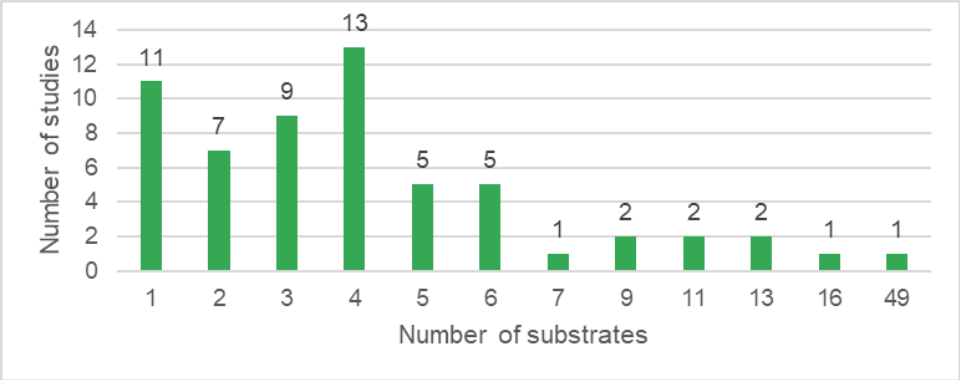


Figure 3.14: Number of substrates in included studies

3.4.3 Inclusion of manure

As a considerable amount of research focused both on BSFL nutrition and waste management, data was collected regarding the inclusion of manure in the rearing substrates (Figure 3.4). Only a small number of studies included manure in the BSFL rearing substrates. Most of the studies that included waste products in the rearing substrates, used

Stellenbosch University https://scholar.sun.ac.za

agricultural or food waste such as fruit and vegetable waste (Galassi *et al.*, 2021; Hoc *et al.*, 2021a).

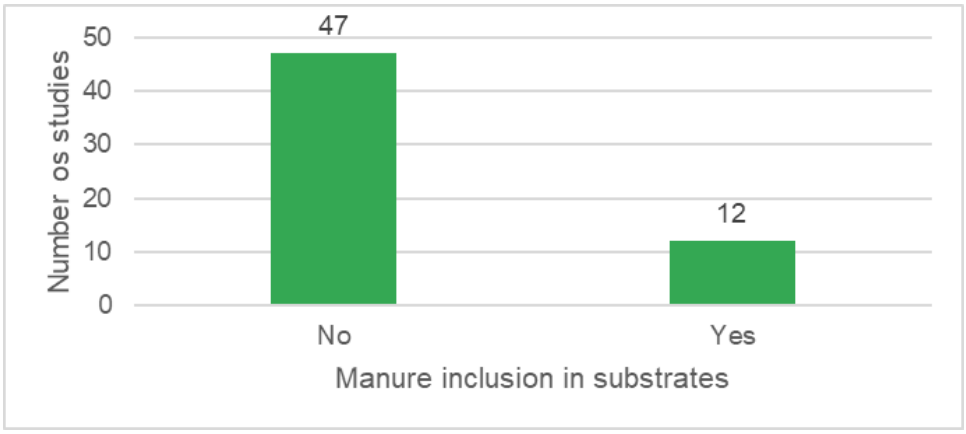


Figure 3.4: Inclusion of manure in rearing substrates in included studies

3.5 Results

The qualitative data regarding the results of the studies constituted the identification of the studies' reported chemical analysis data of both the rearing substrates and the harvested BSFL. Specifically proximate composition and fatty acid profile results were identified. Data regarding other chemical composition analysis such as amino acid profiles were not taken into account as this did not fall within the scope of the review. Along with these data, it was also noted if the studies provided information regarding the formulation of the rearing substrates.

3.5.1 Rearing substrate formulation

The formulation and composition data regarding the rearing substrates was only collected for the studies that conducted randomised control trials, which were 59 in total. Out of these studies, 55 reported the rearing substrate formulation (Figure 3.5). This was expected as the focus of these studies were the effect of nutrition, in which the formulation and ingredients of the rearing substrate play a major role.

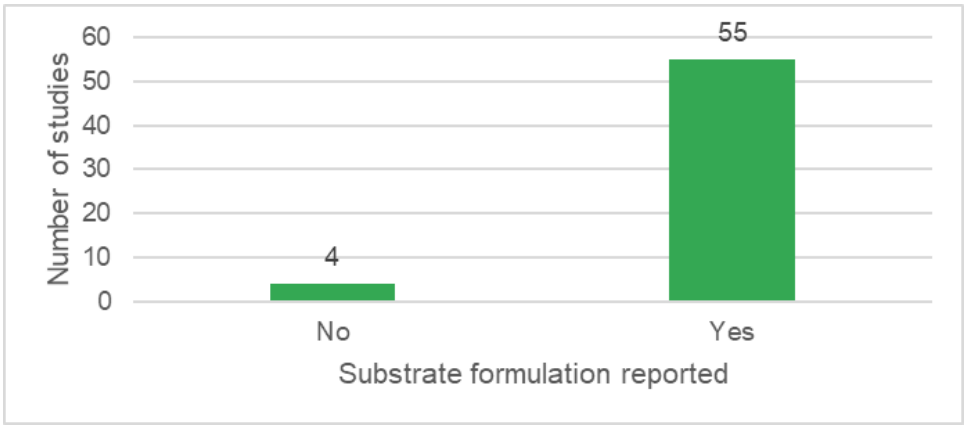


Figure 3.5: Study reporting of rearing substrate formulation

3.5.2 Rearing substrate proximate composition

Fewer studies reported the proximate composition of the rearing substrate (Figure 3.6). Some of the studies that did not report the full proximate composition, did still report the lipid content (Saadoun *et al.*, 2020). This indicated that the focus of the studies was mainly on the effect of specific ingredients in the rearing substrate, and not necessarily the chemical composition of the substrates. This could be a blind spot in the research as studies have shown that the proximate composition, especially the lipid and carbohydrate content of the rearing substrate play a major role in the fatty acid accumulation and synthesis in BSFL and should be taken into account when interpreting trial results (Schreven *et al.*, 2021).

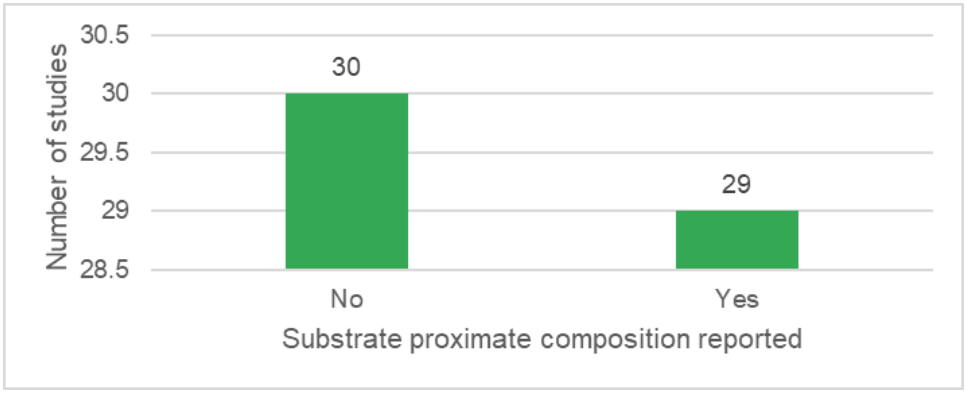


Figure 3.6: Study reporting of rearing substrate proximate composition

3.5.3 Rearing substrate fatty acid profile

Even less of the studies reported the rearing substrate fatty acid composition (Figure 3.7). This is most likely due to the same reason as the lack of proximate composition reporting. Interestingly however, more of the recently published studies included fatty acid analysis of

Stellenbosch University https://scholar.sun.ac.za

the rearing substrate. This is an indication that the importance of the substrate fatty acid profile and its influence on the BSFL is becoming more widely known and investigated.

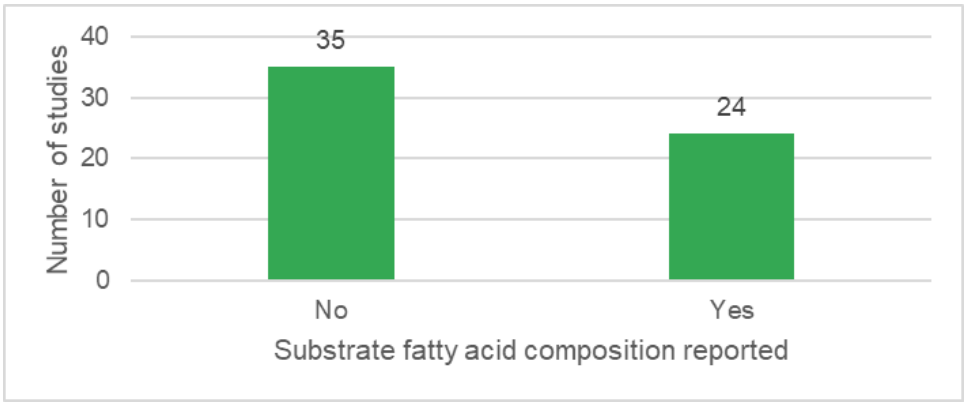


Figure 3.7: Study reporting of rearing substrate fatty acid profile

3.5.4 Black soldier fly larvae proximate composition

The BSFL proximate composition and fatty acid profile data were recorded for the studies that conducted randomised control trials and those that acquired BSFL samples from rearing companies. If the studies only reported the lipid content it was not seen as a proximate composition, however, if they reported both the lipid and the protein content it was noted as a proximate composition. Most of the studies reported the BSFL proximate composition (Figure 3.). Those that did not, focused specifically on the fatty acid profile and therefore only reported the lipid content.

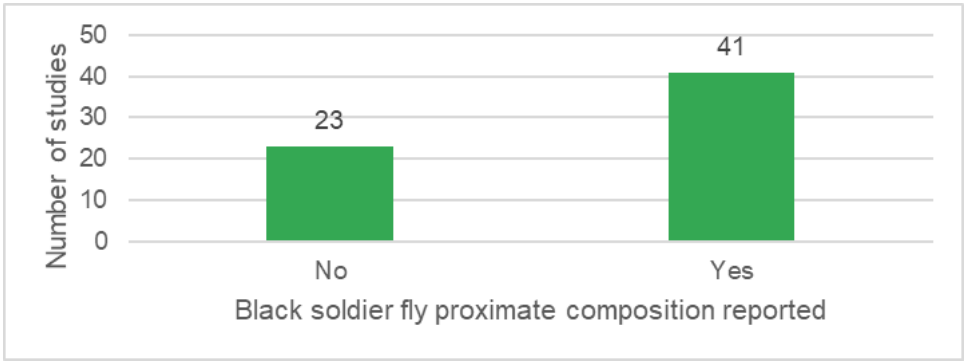


Figure 3.19: Study reporting of black soldier fly larvae proximate composition

Stellenbosch University https://scholar.sun.ac.za

3.5.5 Black soldier fly larvae fatty acid profile

Out of the 64 studies (randomised control trial studies and studies that bought samples), 57 reported the fatty acid profiles of the BSFL (Figure 3.8). The remaining seven studies that did not report the fatty acid profile were still included in this review as it was decided that they contained information that could contribute to the understanding of BSFL fatty acid composition.

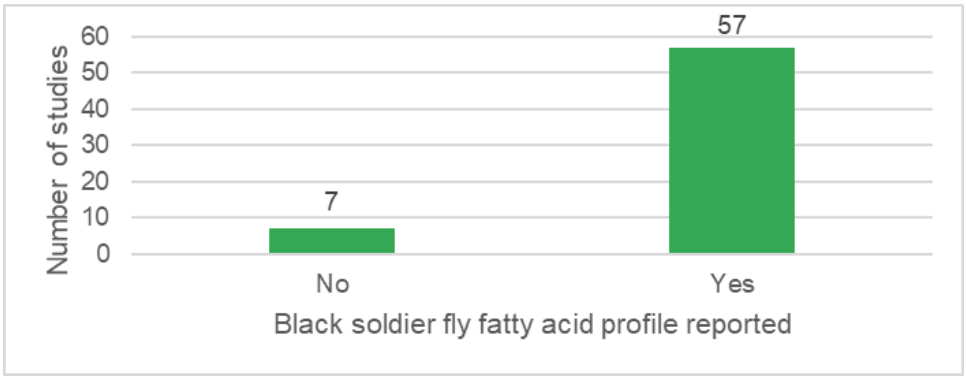


Figure 3.8: Study reporting of black soldier fly larvae fatty acid profile

3.6 Inclusion in meta-analyses

After the examination of each study, a decision was made as to whether to include each individual study in the meta-analysis aspect of this review. A list of criteria used to determine each study's inclusion in the meta-analyses were:

- Did the study report the fatty acid profile of the black soldier fly larvae?
- Did the study have two or more intervention groups?
- Were the fatty acid profile results reported in a compatible format to the meta-analysis?
- Were the fatty acid profile results reported in a compatible unit of measure (mean ± standard deviation or standard error)?

For inclusion in the meta-analyses, the study had to answer yes to all four questions. After the evaluation was complete, there were 26 studies that were selected to be included in the meta-analyses (Figure 3.9). Three of these studies reported two trials each and therefore represented two sets of data. Due to this there were 29 sets of fatty acid data that were included in the meta-analyses.

Stellenbosch University https://scholar.sun.ac.za

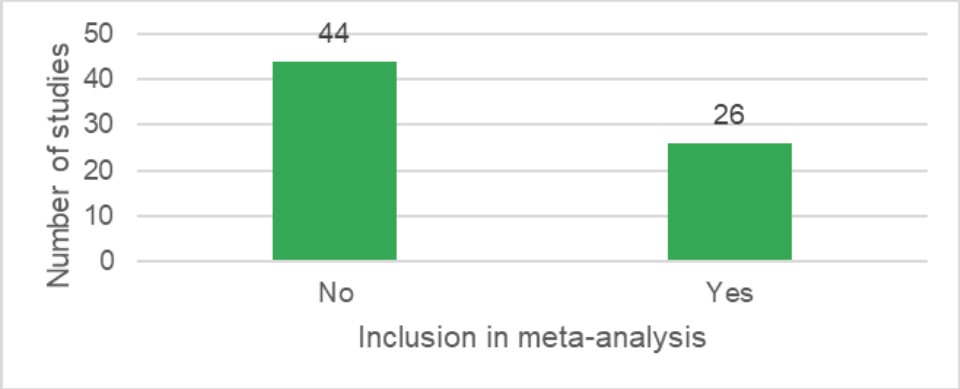


Figure 3.9: Study inclusion in meta-analyses based on criteria

3.7 References

Abduh, M.Y., Nadia, M.H., Syaripudin, Manurung, R. & Putra, R.E., 2018. Factors affecting the bioconversion of Philippine tung seed by black soldier fly larvae for the production of protein and oil-rich biomass. J. Asia Pac. Entomol. 21, 836-842. https://doi.org/10.1016/j.aspen.2018.06.007

Alifian, M.D., Sholikin, M.M., Evvyernie, D. & Nahrowi., 2019. Potential Fatty acid composition of *Hermetia illucens* oil reared on different substrates. in IOP Conf. Ser.: Mater. Sci. Eng. 546. http://doi.org/10.1088/1757-899X/546/6/062002

Barbi, S., Macavei, L.I., Fuso, A., Luparelli, A.V, Caligiani, A., Ferrari, A.M., Maistrello, L. & Montorsi, M., 2020. Valorization of seasonal agri-food leftovers through insects. Sci. Total Environ. 709, 136209. https://doi.org/10.1016/j.scitotenv.2019.136209

Barragan-Fonseca, K.B., Dicke, M. & van Loon, J.J.A., 2017. Nutritional value of the black soldier fly (*Hermetia illucens* L.) and its suitability as animal feed - a review. J. Insects as Food Feed. 3, 105-120. doi.org/10.3920/JIFF2016.0055

Barroso, F.G., Sanchez-Muros, M.J., Rincon, M.A., Rodriguez-Rodriguez, M., Fabrikov, D., Morote, E. & Guil-Guerrero, J.L., 2019. Production of n-3-rich insects by bioaccumulation of fishery waste. J. Food Compos. Anal. 82, 103237. https://doi.org/10.1016/j.jfca.2019.103237

Barroso, F.G., Sánchez-Muros, M., Segura, M., Morote, E., Torres, A., Ramos, R. & Guil, J.L., 2017. Insects as food: enrichment of larvae of *Hermetia illucens* with omega 3 fatty acids by means of dietary modifications. J. Food Compos. Anal. 62, 8-13. https://doi.org/10.1016/j.jfca.2017.04.008

Stellenbosch University https://scholar.sun.ac.za

Bbosa, T., Ndagire, C.T., Mukisa, I.M., Fiaboe, K.K.M. & Nakimbugwe, D., 2019. Nutritional characteristics of selected insects in Uganda for use as alternative protein sources in food and feed. J. Insect Sci. 19, 1-8. https://doi.org/10.1093/jisesa/iez124

Benzertiha, A., Kierończyk, B., Rawski, M., Mikołajczak, Z., Urbański, A., Nogowski, L. & Józefiak, D., 2020. Insect fat in animal nutrition - a review. Ann. Anim. Sci. 20, 1217-1240. https://doi.org/10.2478/aoas-2020-0076

Caligiani, A., Marseglia, A., Leni, G., Baldassarre, S., Maistrello, L., Dossena, A. & Sforza, S., 2018. Composition of black soldier fly prepupae and systematic approaches for extraction and fractionation of proteins, lipids and chitin. Food Res. Int. 105, 812-820. https://doi.org/10.1016/j.foodres.2017.12.012

Caligiani, A., Marseglia, A., Sorci, A., Bonzanini, F., Lolli, V., Maistrello, L. & Sforza, S., 2019. Influence of the killing method of the black soldier fly on its lipid composition. Food Res. Int. 116, 276-282. https://doi.org/10.1016/j.foodres.2018.08.033

Campbell, M., Ortuño, J., Stratakos, A. C., Linton, M., Corcionivoschi, N., Elliot, T., Koidis, A. & Theodoridou, K., 2020. Impact of thermal and high-pressure treatments on the microbiological quality and in vitro digestibility of black soldier fly (*Hermetia illucens*) larvae. Animals. 10, 682. https://doi.org/10.3390/ani10040682

Cullere, M., Woods, M.J., van Emmenes, L., Pieterse, E., Hoffman, L.C. & Dalle Zotte, A., 2019. *Hermetia illucens* larvae reared on different substrates in broiler quail diets: effect on physicochemical and sensory quality of the quail meat. Animals. 9, 525. https://doi.org/10.3390/ani9080525

Danielie, P.P., Lussiana, C., Gasco, L., Amici, A. & Ronchi, B., 2019. The effect of diet formulation on the yield, proximate composition, and fatty acid profile of the black soldier fly (*Hermetia illucens* L.) prepupae intended for animal feed. Animals. 9, 178. doi:10.3390/ani9040178

Egnew, N., Romano, N., Fischer, H. & Sinha, A.K., 2021. Purging black soldier fly larvae (*Hermetia illucens*) compromises their nutritive value as a feedstuff. Int. J. Trop. Insect Sci. https://doi.org/10.1007/s42690-021-00491-x

El-Dakar, M.A., Ramzy, R.R. & Ji, H., 2021a. Influence of substrate inclusion of quail manure on the growth performance, body composition, fatty acid and amino acid profiles of black soldier fly larvae (*Hermetia illucens*). Sci. Total Environ. 772, 145528. https://doi.org/10.1016/j.scitotenv.2021.145528

El-Dakar, M.A., Ramzy, R.R., Ji, H. & Plath, M., 2020. Bioaccumulation of residual omega-3 fatty acids from industrial *Schizochytrium* microalgal waste using black soldier fly (*Hermetia illucens*) larvae. J. Clean. Prod. 268, 122288. https://doi.org/10.1016/j.jclepro.2020. 122288

Stellenbosch University https://scholar.sun.ac.za

El-Dakar, M.A., Ramzy, R.R., Plath, M. & Ji, H., 2021b. Evaluating the impact of bird manure vs. mammal manure on *Hermetia illucens* larvae. J. Clean. Prod. 278, 123570. https://doi.org/10.1016/j.jclepro.2020.123570

El-Dakar, M.A., Ramzy, R.R., Wang, D. & Ji, H., 2021c. Sustainable management of Se-rich silkworm residuals by black soldier flies larvae to produce a high nutritional value and accumulate ω-3 PUFA. Waste Manag. 124, 72-81. https://doi.org/10.1016/j.wasman.2021.01.040

Erbland, P., Alyokhin, A., Perkins, L.B. & Peterson, M., 2020. Dose-dependent retention of omega-3 fatty acids by black soldier fly larvae (Diptera: Stratiomyidae). J. Econ. Entomol. 113, 1221-1226. https://doi.org/10.1093/jee/toaa045

Ewald, N., Vidakovic, A., Langeland, M., Kiessling, A., Sampels, S. & Lalander, C., 2020. Fatty acid composition of black soldier fly larvae (*Hermetia illucens*) - possibilities and limitations for modification through diet. Waste Manag. 102, 40-47 https://doi.org/10.1016/j.wasman.2019.10.014

Fischer, H., Roomano, N. & Sinha, A.K., 2021. Conversion of spent coffee and donuts by black soldier fly (*Hermetia illucens*) larvae into potential resources for animal and plant farming. Insects. 12, 332. https://doi.org/10.3390/insects12040332

Galassi, G., Jucker, C., Parma, P., Lupi, D., Crovetto, G.M., Savoldelli, S. & Colombini, S., 2021. Impact of Agro-industrial byproducts on bioconversion, chemical composition, in vitro digestibility, and microbiota of the black soldier fly (Diptera: Stratiomyidae) larvae. J. Insect Sci. 21, 1-10. https://doi.org/10.1093/jisesa/ieaa148

Gao, Z., Wang, W., Lu, X., Zhu, F., Liu, W., Wang, X. & Lei, C., 2019. Bioconversion performance and life table of black soldier fly (*Hermetia illucens*) on fermented maize straw. J. Clean. Prod. 230, 974-980 https://doi.org/10.1016/j.jclepro.2019.05.074

Giannetto, A., Oliva, S., Ceccon Lanes, C.F., de Araújo Pedron, F., Savastano, D., Baviera, C., Parrino, V., Lo Paro, G., Spano, N.C., Cappello, T., Maisano, M., Mauceri, A. & Fasulo, S., 2020. *Hermetia illucens* (Diptera: Stratiomydae) larvae and prepupae: biomass production, fatty acid profile and expression of key genes involved in lipid metabolism. J. Biotechnol. 307, 44-54 https://doi.org/10.1016/j.jbiotec.2019.10.015

Grossule, V., Vanin, S. & Lavagnolo, M.C., 2020. Potential treatment of leachate by *Hermetia illucens* (Diptera, Stratyomyidae) larvae: performance under different feeding conditions. Waste Manag. Res. 38, 537-545. https://doi.org/10.1177/0734242X19894625

Guil-Guerrero, J.L., Sánchez-Muros, M.J., Fabrikov, D., Rodríguez-Lozano, B., González-Fernández, M.J., Lyashenko, S. & Barroso, F.G., 2020. *Hermetia illucens* larvae as a living bioreactor for simultaneous food by-products recycling and useful oil production. J. Am. Oil Chem. Soc. 97, 717-727. https://doi.org/10.1002/aocs.12370

Stellenbosch University https://scholar.sun.ac.za

Hoc, B., Francis, F., Carpentier, J., Mostade, L., Blecker, C., Purcaro, G. & Caparros Megido, R., 2021a. ω3-enrichment of *Hermetia illucens* (L. 1758) prepupae from oilseed byproducts. J. Saudi Soc. Agric. Sci. 20, 155-163. https://doi.org/10.1016/j.jssas.2021.01.001

Hoc, B., Genva, M., Fauconnier, M-L., Lognay, G., Francis, F. & Megido, R.C., 2020. About lipid metabolism in *Hermetia illucens* (L. 1758): on the origin of fatty acids in prepupae. Sci. Rep. 10, 11916. https://doi.org/10.1038/s41598-020-68784-8

Hoc, B., Tomson, T., Malumba, P., Blecker, C., Jijakli, M.H., Purcaro, G., Francis, F. & Caparros Megido, R., 2021b. Production of rainbow trout (*Oncorhynchus mykiss*) using black soldier fly (*Hermetia illucens*) prepupae-based formulations with differentiated fatty acid profiles. Sci. Total Environ. 794, 148647. https://doi.org/10.1016/j.scitotenv.2021.148647

Jucker, C., Erba, D., Leonardi, M.G., Lupi, D. & Savoldelli, S., 2017. Assessment of vegetable and fruit substrates as potential rearing media for *Hermetia illucens* (Diptera: Stratiomyidae) larvae. Environ. Entomol. 46, 1415-1423. https://doi.org/10.1093/ee/nvx154

Jucker, C., Lupi, D., Moore, C.D., Leonardi, M.G. & Savoldelli, S., 2020. Nutrient recapture from insect farm waste: bioconversion with *Hermetia illucens* (L.) (Diptera: Stratiomyidae). Sustainability. 12, 362. https://doi.org/10.3390/su12010362

Kawasaki, K., Hashimoto, Y., Hori, A., Kawasaki, T., Hirayasu, H., Iwase, S., Hashizume, A., Ido, A., Miura, C., Miura, T., Nakamura, S., Seyama, T., Matsumoto, Y., Kasai, K. & Fujitani, Y., 2019. Evaluation of black soldier fly (*Hermetia illucens*) larvae and pre-pupae raised on household organic waste, as potential ingredients for poultry feed. Animals. 9, 98. https://doi.org/10.3390/ani9030098

Kierończyk, B., Sypniewski, J., Rawski, M., Czekala, W., Świątkiewicz, S. & Józefiak, D., 2020. From waste to sustainable feed material: the effect of *Hermetia illucens* oil on the growth performance, nutrient digestibility, and gastrointestinal tract morphometry of broiler chickens. Ann. Anim. Sci. 20, 157-177 https://doi.org/10.2478/aoas-2019-0066

Koutsos, L., McComb, A. & Finke, M., 2019. Insect composition and uses in animal feeding applications: a brief review. Ann. Entomol. Soc. Am. 112, 544-551. https://doi.org/10.1093/aesa/saz033

Kuppusamy, G., Kong, C.K., Segaran, G.C., Tarmalingam, E., Herriman, M., Ismail, M.F., Khan, T.M., Low, L.E. & Goh, B-H., 2020. Hummingbird-leaves-reared black soldier fly prepupae: assessment of nutritional and heavy metal compositions. Biology. 9, 274. https://doi.org/10.3390/biology9090274

Stellenbosch University https://scholar.sun.ac.za

Leong, S.Y. & Kutty, S.R.M. 2020a. Characteristic of fatty acids biotransform from *Hermetia illucens* prepupae fed with various organic wastes before conversion to methyl ester form. in IOP Conf. Ser.: Mater. Sci. Eng. 736. https://doi.org/10.1088/1757-899X/736/4/042004

Leong, S.Y. & Kutty, S.R.M., 2020b. Characteristic of *Hermetia illucens* fatty acid and that of the fatty acid methyl ester synthesize based on upcycling of perishable waste. Waste Biomass Valorization. 11, 5607-5614. https://doi.org/10.1007/s12649-020-01018-0

Leong, S.Y., Kutty, S.R.M., Tan, C.K. & Tey, L.H., 2015. Comparative study on the effect of organic waste on lauric acid produced by *Hermetia illucens* larvae via bioconversion. J. Eng. Sci. Technol. 10, 52–63.

Li, T., Higgins, J.P.T. & Deeks, J.J. (editors), 2019. Chapter 5: Collecting data. In: Cochrane handbook for systematic reviews of interventions. 2nd Edition. Ed: Higgins, J.P.T., Thomas, J., Chandler, J., Cumpston, M., Li, T. and Welch, V.A., John Wiley & Sons, Chichester, England. pp.109-142.

Liland, N.S., Biancarosa, I., Araujo, P, Biemans, D., Bruckner, C.G., Woogbø, R., Torstensen, B.E. & Lock, E.J., 2017. Modulation of nutrient composition of black soldier fly (*Hermetia illucens*) larvae by feeding seaweed-enriched media. PLoS One. 12. https://doi.org/10.1371/journal.pone.0183188

Liu, X., Chen, X., Wang, H., Yang, Q., ur Rehman, K., Li, W., Cai, M., Li, Q., Mazza, L., Zhang, J., Yu, Z. & Zheng, L., 2017. Dynamic changes of nutrient composition throughout the entire life cycle of black soldier fly. PLoS One. 12. https://doi.org/10.1371/journal.pone.0182601

Liu, Z., Minor, M., Morel, P.C.H. & Najar-Rodriguez, A.J., 2018. Bioconversion of three organic wastes by black soldier fly (Diptera: Stratiomyidae) larvae. Environ. Entomol. 47, 1609-1617. https://doi.org/10.1093/ee/nvy141

Matthaeus, B., Piofczyk, T., Katz, H. & Pudel, F., 2019. Renewable resources from insects: exploitation, properties, and refining of fat obtained by cold-pressing from *Hermetia illucens* (black soldier fly) larvae. Eur. J. Lipid Sci. Technol. 121, 1800376. https://doi.org/10.1002/ejlt.201800376

Meneguz, M., Schiavone, A., Gai, F., Dama, A., Lussiana, C., Renna, M. & Gasco, L., 2018. Effect of rearing substrate on growth performance, waste reduction efficiency and chemical composition of black soldier fly (*Hermetia illucens*) larvae. J. Sci. Food Agric. 98, 5776–5784. https://doi.org/10.1002/jsfa.9127

Stellenbosch University https://scholar.sun.ac.za

Mohamad, L., Dina, F., Hasan, H.A., Sudesh, K. & Baidurah, S., 2019. Effect of feeding strategy on the protein and fatty acid contents of black soldier fly prepupae (*Hermetia illucens*) for the potential applications as animal feed and promising alternative protein-rich food. in IOP Conf. Ser.: Mater. Sci. Eng. 716. https://doi.org/10.1088/1757-899X/716/1/012006

Mohd-Noor, S-N., Wong, C-Y., Lim, J-W., Mah-Hussin, M-I-A., Uemura, Y., Lam, M-K., Ramli, A., Bashir, M.J.K. & Tham, L., 2017. Optimization of self-fermented period of waste coconut endosperm destined to feed black soldier fly larvae in enhancing the lipid and protein yields. Renew. Energy. 111, 646-654. https://doi.org/10.1016/j.renene.2017.04.067

Nguyen, T.T.X., Tomberlin, J.K. & Vanlaerhoven, S., 2015. Ability of black soldier fly (Diptera: Stratiomyidae) larvae to recycle food waste. Environ. Entomol. 44, 406-410. https://doi.org/10.1093/ee/nvv002

Nyakeri, E.M., Ogola, H.J.O., Ayieko, M.A. & Amimo, F.A., 2017. Valorisation of organic waste material: growth performance of wild black soldier fly larvae (*Hermetia illucens*) reared on different organic wastes. J. Insects as Food Feed. 3, 193-202. https://doi.org/10.3920/JIFF2017.0004

Oonincx, D.G.A.B., van Broekhoven, S., van Huis, A. & van Loon, J.J.A., 2015. Feed conversion, survival and development, and composition of four insect species on diets composed of food by-products. PLoS One. 10. https://doi.org/10.1371/journal.pone.0144601

Oonincx, D.G.A.B., Laurent, S., Veenenbos, M.E. & van Loon, J.J.A., 2020. Dietary enrichment of edible insects with omega 3 fatty acids. Iinsect Sci. 27, 500-509. https://doi.org/10.1111/1744-7917.12669

Opatovsky, I., Vitenberg, T., Jonas-Levi, A. & Gutman, R., 2021. Does consumption of baker's yeast (*Saccharomyces cerevisiae*) by black soldier fly (Diptera: Stratiomyidae) larvae affect their fatty acid composition? J. Insect Sci. 21, 1-5. https://doi.org/10.1093/jisesa/ieab031

Pang, W., Hou, D., Ke, J., Chen, J., Holtzapple, M.T., Tomberlin, J.K., Chen, H., Zhang, J. & Li, Q., 2020. Production of biodiesel from CO_2 and organic wastes by fermentation and black soldier fly. Renew. Energy 149, 1174-1181. https://doi.org/10.1016/j.renene.2019.10.099

Pastor, B., Velasquez, Y., Gobbi, P. & Rojo, S., 2015. Conversion of organic wastes into fly larval biomass: bottlenecks and challenges. J. Insects as Food Feed. 1, 179-193. https://doi.org/10.3920/JIFF2014.0024

Stellenbosch University https://scholar.sun.ac.za

Rabani, V., Cheatsazan, H. & Davani, S., 2019. Proteomics and lipidomics of black soldier fly (Diptera: Stratiomyidae) and blow fly (Diptera: Calliphoridae) larvae. J. Insect Sci. 19, 29. https://doi.org/10.1093/jisesa/iez050

Riudavets, J., Castañé, C., Agustí, N., del Arco, L., Diaz, I. & Castellari, M., 2020. Development and biomass composition of *Ephestia kuehniella* (Lepidoptera: Pyralidae), *Tenebrio molitor* (Coleoptera: Tenebrionidae), and *Hermetia illucens* (Diptera: Stratiomyidae) reared on different byproducts of the agri-food industry. J. Insect Sci. 20, 17. https://doi.org/10.1093/jisesa/ieaa085

Romano, N., Fischer, H., Kumar, V., Francis, S.A. & Sinha, A.K., 2021. Productivity, conversion ability, and biochemical composition of black soldier fly (*Hermetia illucens*) larvae fed with sweet potato, spent coffee or dough. Int. J. Trop. Insect Sci. https://doi.org/10.1007/s42690-021-00532-5

Saadoun, J.H., Montevecchi, G., Zanasi, L., Bortolini, S., Macavei, L.I., Masino, F., Maistrello, L. & Antonelli, A., 2020. Lipid profile and growth of black soldier flies (*Hermetia illucens*, Stratiomyidae) reared on by-products from different food chains. J. Sci. Food Agric. 100, 3648-3657. https://doi.org/10.1002/jsfa.10397

Scala, A., Cammack, J.A., Salvia, R., Scieuzo, C., Franco, A., Bufo, S.A., Tomberlin, J.K. & Falabella, P., 2020. Rearing substrate impacts growth and macronutrient composition of *Hermetia illucens* (L.) (Diptera: Stratiomyidae) larvae produced at an industrial scale. Sci. Rep. 10, 19448. https://doi.org/10.1038/s41598-020-76571-8

Schreven, S.J.J., Yener, S., van Valenberg, H.J.F., Dicke, M. & van Loon, J.J.A., 2021. Life on a piece of cake: performance and fatty acid profiles of black soldier fly larvae fed oilseed by-products. J. Insects as Food Feed. 7, 35-49. https://doi.org/10.3920/JIFF2020.0004

Shumo, M., Osuga, I.M., Khamis, F.M., Tanga, C.M., Fiaboe, K.K.M., Subramanian, S., Ekesi, S., van Huis, A. & Borgemeister, C., 2019. The nutritive value of black soldier fly larvae reared on common organic waste streams in Kenya. Sci. Rep. 9, 10110. https://doi.org/10.1038/s41598-019-46603-z

Somroo, A.A., ur Rehman, K., Zheng, L., Cai, M., Xiao, X., Hu, S., Mathys, A., Gold, M., Yu, Z. & Zhang, J., 2019. Influence of *Lactobacillus buchneri* on soybean curd residue co-conversion by black soldier fly larvae (*Hermetia illucens*) for food and feedstock production. Waste Manag. 86, 114-122. https://doi.org/10.1016/j.wasman.2019.01.022

Spranghers, T., Ottoboni, M., Klootwijk, C., Ovyn, A., Deboosere, S., De Meulenaer, B., Michiels, J., Eeckhout, M., De Clercq, P. & De Smet, S., 2017. Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates. J. Sci. Food Agric. 97, 2594-2600. https://doi.org/10.1002/jsfa.8081

Stellenbosch University https://scholar.sun.ac.za

St-Hilaire, S., Cranfill, K., McGuire, M.A., Mosley, E.E., Tomberlin, J.K., Newton, L., Sealey, W., Sheppard, C. & Irving, S., 2007. Fish offal recycling by black soldier fly produces a foodstuff high in omega-3 fatty acids. J. World Aquac. Soc. 38, 197-202. https://doi.org/10.1111/j.1749-7345.2007.00101.x

Starcevic, K., Lozica, L., Gavrilovic, A., Heruc, Z. & Masek, T., 2019. Fatty acid plasticity of black soldier fly (*Hermetia Illucens*) larvae reared on alternative feeding media: crude olive cake and processed animal protein. J. Anim. Feed Sci. 28, 374-382. https://doi.org/10.22358/jafs/114434/2019

Surendra, K.C., Olivier, R., Tomberlin, J.K., Jha, R. & Khanal, S.K., 2016. Bioconversion of organic wastes into biodiesel and animal feed via insect farming. Renew. Energy. 98, 197-202. https://doi.org/10.1016/j.renene.2016.03.022

Surendra, K.C., Tomberlin, J.K., van Huis, A., Cammack, J.A., Heckmann, L.L. & Khanal, S.K., 2020. Rethinking organic wastes bioconversion: evaluating the potential of the black soldier fly (*Hermetia illucens* (L.)) (Diptera: Stratiomyidae) (BSF). Waste Manag. 117, 58-80. https://doi.org/10.1016/j.wasman.2020.07.050

Truzzi, C., Giorgini, E., Annibaldi, A., Antonucci, M., Illuminati, S., Scarponi, G., Riolo, P., Isidoro, N., Conti, C., Zarantoniello, M., Cipriani, R. & Olivotto, I., 2020. Fatty acids profile of black soldier fly (*Hermetia illucens*): influence of feeding substrate based on coffee-waste silverskin enriched with microalgae. Anim. Feed Sci. Technol. 259, 114309. https://doi.org/10.1016/j.anifeedsci.2019.114309

Wang, S.Y., Wu, L., Li, B. & Zhang, D., 2020. Reproductive potential and nutritional composition of *Hermetia illucens* (Diptera: Stratiomyidae) prepupae reared on different organic wastes. J. Econ. Entomol. 113, 527-537. https://doi.org/10.1093/jee/toz296

Weru, J., Chege, P. & Kinyuru, J., 2021. Nutritional potential of edible insects: a systematic review of published data. Int. J. Trop. Insect Sci. 41, 2015-2037. https://doi.org/10.1007/s42690-021-00464-0

Wong, C-Y., Rosli, S-S., Uemura, Y., Ho, Y.C., Leejeerajumnean, A., Kiatkittipong, W., Cheng, C-K., Lam, M-K. & Lim, J-W., 2019. Potential protein and biodiesel sources from black soldier fly larvae: insights of larval harvesting instar and fermented feeding medium. Energies. 12, 1570. https://doi.org/10.3390/en12081570

Stellenbosch University https://scholar.sun.ac.za

CHAPTER 4

Meta-analyses of saturated fatty acids

Black soldier flies (*Hermetia illucens*) do not have functional feeding mouth parts during their adult stage and therefore they build up energy reserves in the form of lipids in their larval instars. These energy reserves are then utilised for maintenance and reproduction during their adult stage. Saturated fatty acids (SFAs) play an important role in this as they are less prone to oxidation than unsaturated fatty acids. Due to this the black soldier fly larval (BSFL) fatty acid profile can consist of up to 86.89% SFA (Danieli *et al.*, 2019). In order to understand how the levels of individual SFAs can be affected by the nutritional composition of rearing substrates, meta-analyses were performed on the collected data regarding the concentrations of individual SFAs in the BSFL fatty acid profile. The data included in the meta-analyses came from 29 trials from 26 published studies (Table **A** in Appendix A). Three of these studies reported results from multiple trials and thus contributed multiple trials to the meta-analyses.

Fifteen SFAs were identified to be either always or sometimes present in the BSFL fatty acid profile (Table 4.). Meta-analyses were only performed on data regarding eight of these SFAs. The other SFAs were excluded from meta-analyses as they were not reported as present in an adequate number of studies to perform a meta-analysis.

Stellenbosch University https://scholar.sun.ac.za

Table 4.1: List of saturated fatty acids identified as present in black soldier fly larval fatty acid profile

Common name	Preferred IUPAC name	Omega nomenclature	Inclusion in meta-analysis
Caprylic acid	Octanoic acid	C8:0	No
Capric acid	Decanoic acid	C10:0	Yes
Undecylic acid	Undecanoic acid	C11:0	No
Lauric acid	Dodecanoic acid	C12:0	Yes
Tridecylic acid	Tridecanoic acid	C13:0	No
Myristic acid	Tetradecanoic acid	C14:0	Yes
Pentadecylic acid	Pentadecanoic acid	C15:0	No
Palmitic acid	Hexadecanoic acid	C16:0	Yes
Margaric acid	Heptadecanoic acid	C17:0	Yes
Stearic acid	Octadecanoic acid	C18:0	Yes
Arachidic acid	Eicosanoic acid	C20:0	Yes
Heneicosylic acid	Heneicosanoic acid	C21:0	No
Behenic acid	Docosanoic acid	C22:0	No
Tricosylic acid	Tricosanoic acid	C23:0	No
Lignoceric acid	Tetracosanoic acid	C24:0	No

4.1 Summary statistics

Summary statistics regarding the individual saturated fatty acids (SFAs) were collected and are shown in Table 4.2. The table shows the lowest and highest concentrations of the individual SFAs among all the included trials. A number of the SFAs were found to be absent in the black soldier fly larval (BSFL) fatty acid profile and in those cases the lowest concentration was denoted as zero. Four of the SFAs were found to invariably be present in the BSFL fatty acid profile. These SFAs were lauric acid, myristic acid, palmitic acid and stearic acid. The highest observed concentrations of the different SFAs were found to vary considerably. Lauric acid was noted to be the SFA with the highest observed concentration (68.00% of the total fatty acids), which was more than 20% higher than any of the other

Stellenbosch University https://scholar.sun.ac.za

SFAs (Jucker *et al.*, 2017). The total SFA content in terms of the BSFL fatty acid profile was found to range from 28.11% (Starcevic *et al.*, 2019) to 86.89% (Danieli *et al.*, 2019). The differences in the concentration of the individual fatty acids due to rearing substrate nutritional composition reported by the different trials were also investigated and the largest difference found for each of the SFAs is also reported in Table 4.2. The largest detected difference in concentration was found for lauric acid with a difference of almost 50% (Guil-Guerrero *et al.*, 2020). The total SFA content and thus the level of saturation of the BSFL fatty acid profile was found to be differ by up to 37.99% (Starcevic *et al.*, 2019).

Table 4.2: Summary statistics regarding levels of individual saturated fatty acids (SFAs) in black soldier fly larval fatty acid profile (in % of total fatty acids)

Fatty acid	Lower concentration limit (mean ± SD)	Higher concentration limit (mean ± SD)	Largest change due to substrate composition (mean [CI])
Capric acid (C10:0)	0 *	3.80 ± 0.30 (Guil-Guerrero *et al.*, 2020)	3.40 [3.04,3.76] (Guil-Guerrero *et al.*, 2020)
Lauric acid (C12:0)	6.94 ± 2.39 (Schreven *et al.*, 2021)	68.00 ± 0.10 (Jucker *et al.*, 2017)	49.80 [47.35,52.25] (Guil-Guerrero *et al.*, 2020)
Myristic acid (C14:0)	1.79 ± 0.44 (Schreven *et al.*, 2021)	16.70 ± 1.20 (Guil-Guerrero *et al.*, 2020)	12.70 [11.27,14.13] (Guil-Guerrero *et al.*, 2020)
Palmitic acid (C16:0)	6.77 ± 0.28 (Hoc *et al.*, 2021a)	24.85 ± 3.33 (Romano *et al.*, 2021)	13.50 [12.25,14.75] (Guil-Guerrero *et al.*, 2020)
Margaric acid (C17:0)	0 *	2.93 ± 0.40 (Gao *et al.*, 2019)	0.50 [0.26,0.74] (Oonincx *et al.*, 2015)
Stearic acid (C18:0)	0.09 ± 0.10 (Jucker *et al.*, 2017)	21.40 ± 0.90 (Truzzi *et al.*, 2020)	16.70 [16.15,17.25] (Truzzi *et al.*, 2020)
Arachidic acid (C20:0)	0 *	11.00 ± 0.30 (Truzzi *et al.*, 2020)	9.80 [9.64,9.96] (Truzzi *et al.*, 2020)
Behenic acid (C22:0)	0 *	16.00 ± 0.40 (Truzzi *et al.*, 2020)	15.50 [15.21,15.79] (Truzzi *et al.*, 2020)
Total SFA	28.11 ± 2.18 (Starcevic *et al.*, 2019)	86.89 ± 0.57 (Danieli *et al.*, 2019)	37.99 [33.3,42.68] (Starcevic *et al.*, 2019)

* Fatty acid was absent in black soldier fly larvae in some included trials.

All values presented as % of total fatty acids.

SD – standard deviation.

CI – confidence interval.

Stellenbosch University https://scholar.sun.ac.za

Data collected from each trial for the purpose of the meta-analyses were the concentrations of the individual fatty acids in respect to the BSFL fatty acid profile (mean ± standard deviation) and the fatty acid profile analysis sample sizes. Some of the studies reported the results as mean ± standard error. In those cases the standard deviation were calculated using Equation 4.1.

Equation 4.1: Standard deviation equation

$$Standard\ deviation = standard\ error\ \times \sqrt{sample\ size}$$

After a preliminary meta-analysis was performed on the collected data, it was found that the data was too heterogenous for the analysis to convey accurate results. Therefore the data were transformed by means of the Freeman-Tukey double arcsine transformation (Freeman & Tukey, 1950). This transformation can be seen in Equation 4..

Equation 4.2: Freeman-Tukey double arcsine transformation

$$\hat{y}_i\ = \arcsin\sqrt{y_i/(100+1)} + \ arcsin\sqrt{(y_i+1)/(100+1)}\,,$$

where y_i is the original data point and $\hat{y}_i$ is the transformed data point. The equation above is the original formula proposed in the article by Freeman and Tukey. The two arcsine values can however also be divided by two with the purpose of having the transformed values on the same scale as the arcsine-square-root transformation, which is a different transformation utilised for percentage data transformation. The meta-analyses were then performed on the transformed data, which resulted in the calculated intervention effects depicted in the form of forest plots and the average intervention effect.

The Z-test was performed to evaluate the overall effect, where the null hypothesis was that the observed average difference in means is purely due to chance and the alternative hypothesis that there was indeed an effect. The effect being the difference in the individual SFA concentration due to changes in the rearing substrate composition. The Chi^2 test was performed to evaluate if there was heterogeneity among the intervention effects observed by the different trials. The null hypothesis of the Chi^2 test was that the intervention effects are homogeneous, and the alternative hypothesis was that there was heterogeneity among the intervention effects. The extent of the heterogeneity was evaluated by means of the I^2 statistic and the Tau^2 statistic. The I^2 statistic represents the percentage of heterogeneity

Stellenbosch University https://scholar.sun.ac.za

among the observed intervention effects. The Tau^2 statistic represents the variance among the observed intervention effects and is taking into account by the meta-analysis software when the trials are weighted.

4.2 Capric acid

Capric acid is a medium chain saturated fatty acid that consists of a 10 carbon-chain (C10:0). It occurs naturally in coconut and palm kernel oil (Kenar *et al.*, 2017). It was reported to be present in the black soldier fly larval (BSFL) fatty acid profile by 20 of the included trials. Depicted in Table 4.2 it can be seen that the highest reported concentration of capric acid was 3.80% ± 0.30 (Guil-Guerrero *et al.*, 2020) and the largest observed difference in concentration, in meta-analysis referred to as the difference in means of the intervention effect size, reported by a trial was 3.40% (Guil-Guerrero *et al.*, 2020). The effect size seems to be relatively low compared to that found for the other saturated fatty acids (SFAs).

The meta-analysis of the capric acid data can be seen in Figure 4.. The Chi^2 test indicated that there was no heterogeneity among the intervention effects (P = 1.00). The Tau^2 statistic and the I^2 statistic both also indicated that the intervention effects were homogeneous and could therefore be accurately compared through the meta-analysis. The Z-test for the overall effect suggested that the rearing substrate had a significant effect on the capric acid concentration of the larvae. As the meta-analysis was performed on the transformed data, the average effect size reported is on a different scale to that of the collected data. This is also the case for the meta-analyses performed on the data regarding the other fatty acids. The average effect sizes of the different meta-analyses can therefore not be interpreted in terms of the original scale (as percentage of total fatty acids), but can however be compared to each other. The average effect sizes will be discussed later in this chapter.

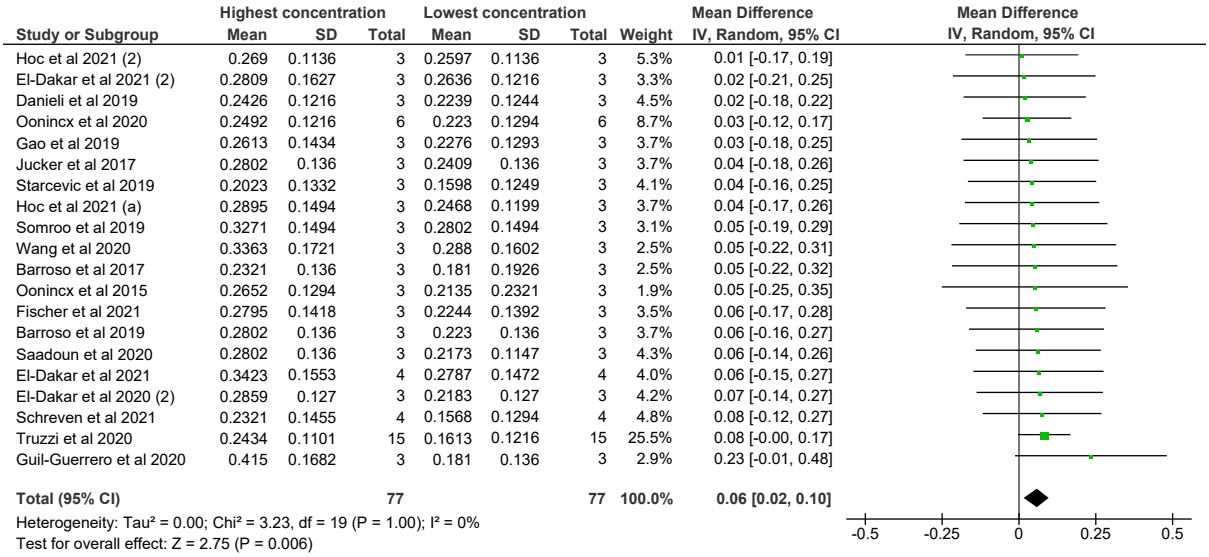


Figure 4.1: Software output of meta-analysis of capric acid concentration of black soldier fly larval fatty acid profile

4.3 Lauric acid

Lauric acid is a medium chain SFA that consists of a 12 carbon-chain (C12:0). Lauric acid is typically found in high concentrations in coconut and palm kernel oil (45-55%) (Kenar *et al.*, 2017). Black soldier fly larvae (BSFL) have the ability to biosynthesize lauric acid from other fatty acids and other sources of energy such as carbohydrates (Hoc *et al.*, 2020). Studies have shown that lauric acid is one of the major fatty acids found in black soldier fly larvae. Therefore, it was unsurprising that all 29 included trials reported its presence in the BSFL fatty acid profile. Table 4.2 shows that the lowest reported concentration of lauric acid was 6.94% ± 2.39 when the larvae were reared on *crambe* pressed cake, which is an oilseed by-product and is characterized by having a highly unsaturated fatty acid profile (Schreven *et al.*, 2021). The highest observed concentration was 68.00% ± 0.10 (Jucker *et al.*, 2017). In this case the larvae were reared on fruit, which mostly consists of carbohydrates and is low in lipids. This reiterates the larvae's reported ability to synthesize lauric acid from other forms of energy. The largest identified difference in lauric acid concentration was 49.80% (Guil-Guerrero *et al.*, 2020). This shows the major effect that the rearing substrate composition can have on the lauric acid content of the larvae.

Figure 4.10 depicts the meta-analysis and resulting forest plot of the lauric acid data. The Chi^2 test shows that there was heterogeneity among the studies (P = 0.002) and the I^2 statistic indicates a moderate level of heterogeneity (49%). The heterogeneity was sufficiently low that it would not significantly influence the meta-analysis. The heterogeneity

Stellenbosch University https://scholar.sun.ac.za

should however be taken into account when interpreting the results of the meta-analysis as it has an effect on the accuracy of the calculated average effect size. The forest plot suggests that the heterogeneity might have originated from one trial (Guil-Guerrero *et al.*, 2020) as there is less overlap of its confidence interval with the other studies. For the lauric acid meta-analysis the Tau^2 was calculated as 0.04. This measure of variation among the studies is taken into account when the individual studies are weighted. The Z-test for the overall effect resulted in a very small P-value (P < 0.0001). This suggested that the rearing substrate composition had a significant effect on the lauric acid concentration of the larvae.

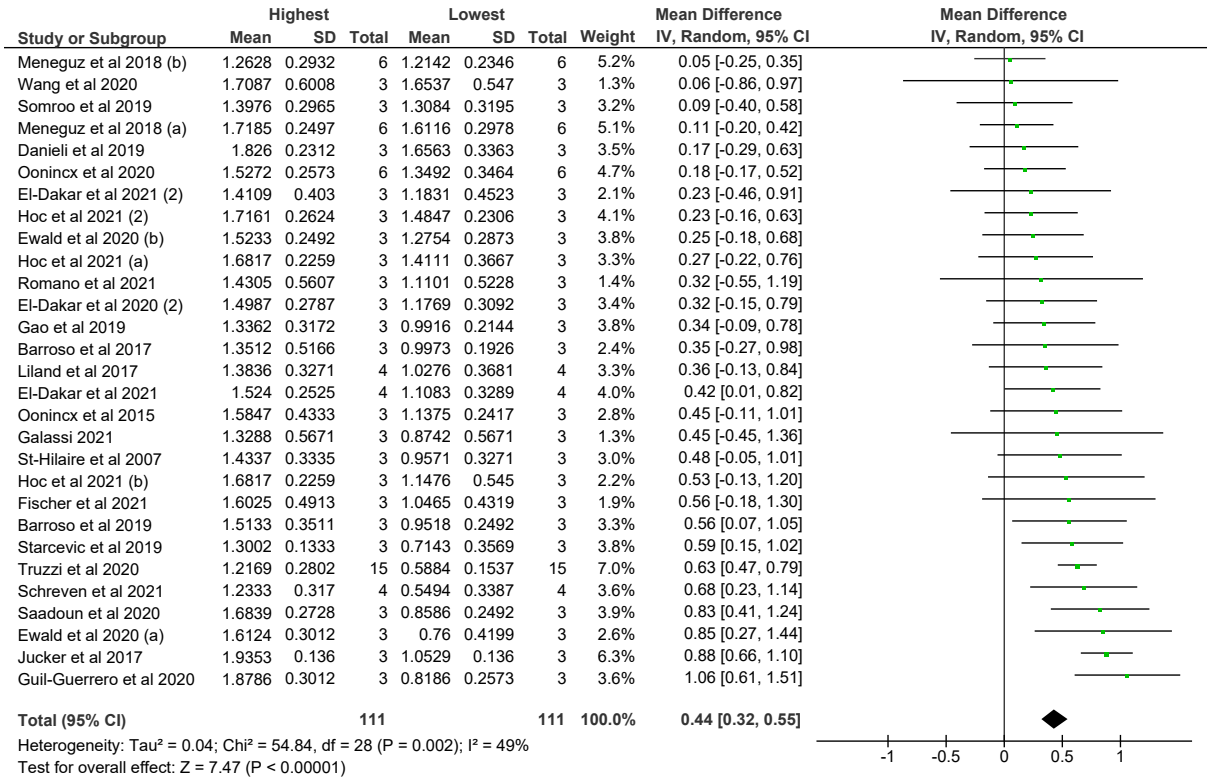


Figure 4.10: Software output of meta-analysis of lauric acid concentration of black soldier fly larval fatty acid profile

4.4 Myristic acid

Myristic acid is a medium chain saturated fatty acid (C14:0). It is found in many plant oils, such as coconut and palm, and also in pork lard and beef tallow (Kenar *et al.*, 2017). Myristic acid is another fatty that was identified as always being present in the BSFL fatty acid profile, however generally in smaller quantities than lauric acid. As shown in Table 4.2 the lowest observed concentration of myristic acid was 1.79% ± 0.44 when the larvae were reared on *crambe* pressed cake (Schreven *et al.*, 2021) and the highest observed concentration was 16.70% ± 1.20 when the larvae were reared on coconut by-product (Guil-Guerrero *et al.*,

2020). These concentrations indicate an association between the concentration of myristic acid in the rearing substrate and the BSFL fatty acid profile. The largest difference in means was identified as 12.70% (Guil-Guerrero *et al.*, 2020). This largest intervention effect was lower compared to many of the other identified SFAs.

The meta-analysis performed on the myristic acid concentration data is shown in Figure 4.. The tests for heterogeneity indicated that there was no detectable level of heterogeneity of importance as the Chi^2 test had a high P-value (P = 0.98) and the I^2 statistic was 0%. The forest plot also illustrates general overlap of the various confidence intervals. Therefore, the data from the difference trials were sufficiently homogeneous to be comparable in terms of a meta-analysis. The Z-test for the overall effect indicated that the rearing substrate had a significant effect on the myristic acid concentration (P < 0.00001).

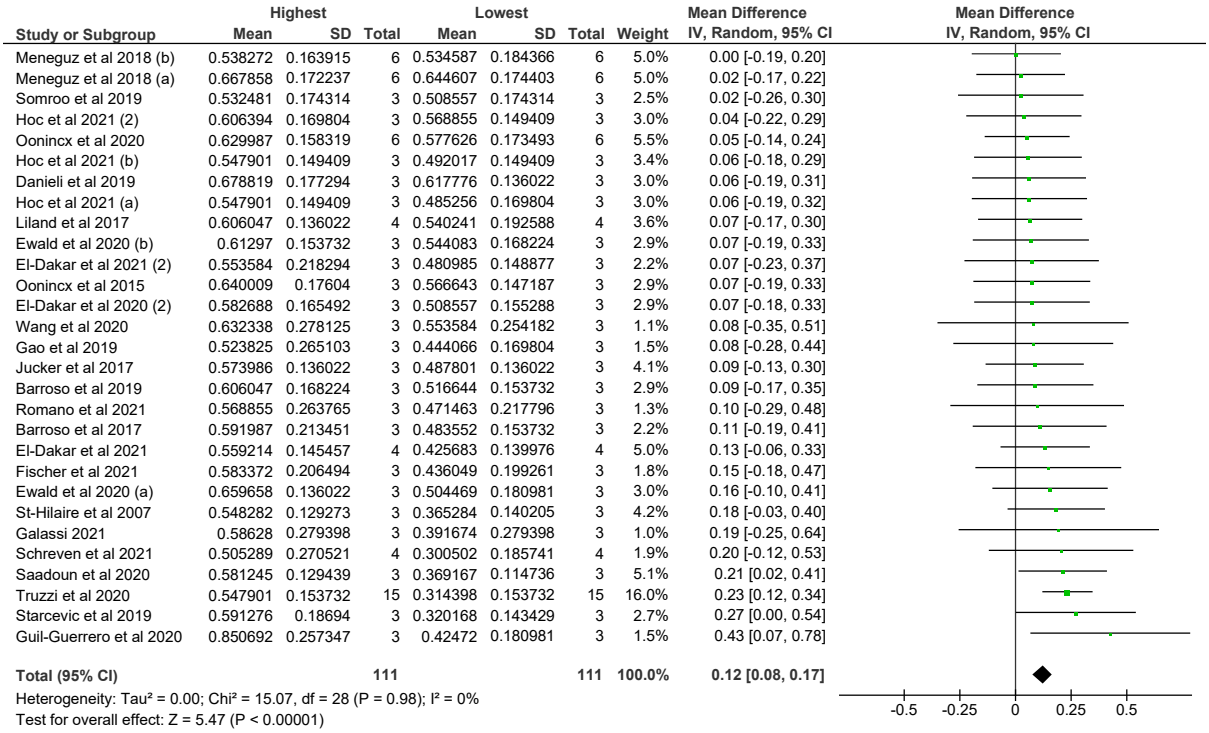


Figure 4.3: Software output of meta-analysis of myristic acid concentration of black soldier fly larval fatty acid profile

4.5 Palmitic acid

Palmitic acid is a 16 carbon chain fatty acid (C16:0) and is the most common saturated fatty acid found in animal fats, plant oils and microorganisms (Kenar *et al.*, 2017). As with lauric acid and myristic acid, palmitic acid was found to consistently be present in the BSFL fatty acid profile as it was found in all 29 of the included trials. Table 4.2 shows that the lowest

Stellenbosch University https://scholar.sun.ac.za

observed concentration of palmitic acid was 6.77% ± 0.28 (Hoc *et al.*, 2021a). This low concentration was observed when the larvae were reared on rapeseed oil cake, which has a low level of saturation. The highest observed concentration was 24.85% ± 3.33 when the larvae were reared on spent coffee (Romano *et al.*, 2021). The largest identified difference in means was 13.50% (Guil-Guerrero *et al.*, 2020).

Figure 4. shows the results of the meta-analysis performed on the palmitic acid concentration data. The statistical tests for heterogeneity indicated that there was no observable heterogeneity as the Chi^2 test had a very high P-value (0.99), $Tau^2 = 0$ and $I^2 = 0\%$. Therefore, the effect sizes of the different trials were comparable. The Z-test for the overall effect indicated that the rearing substrate had a significant effect on the palmitic acid concentration of the larvae ($P < 0.00001$).

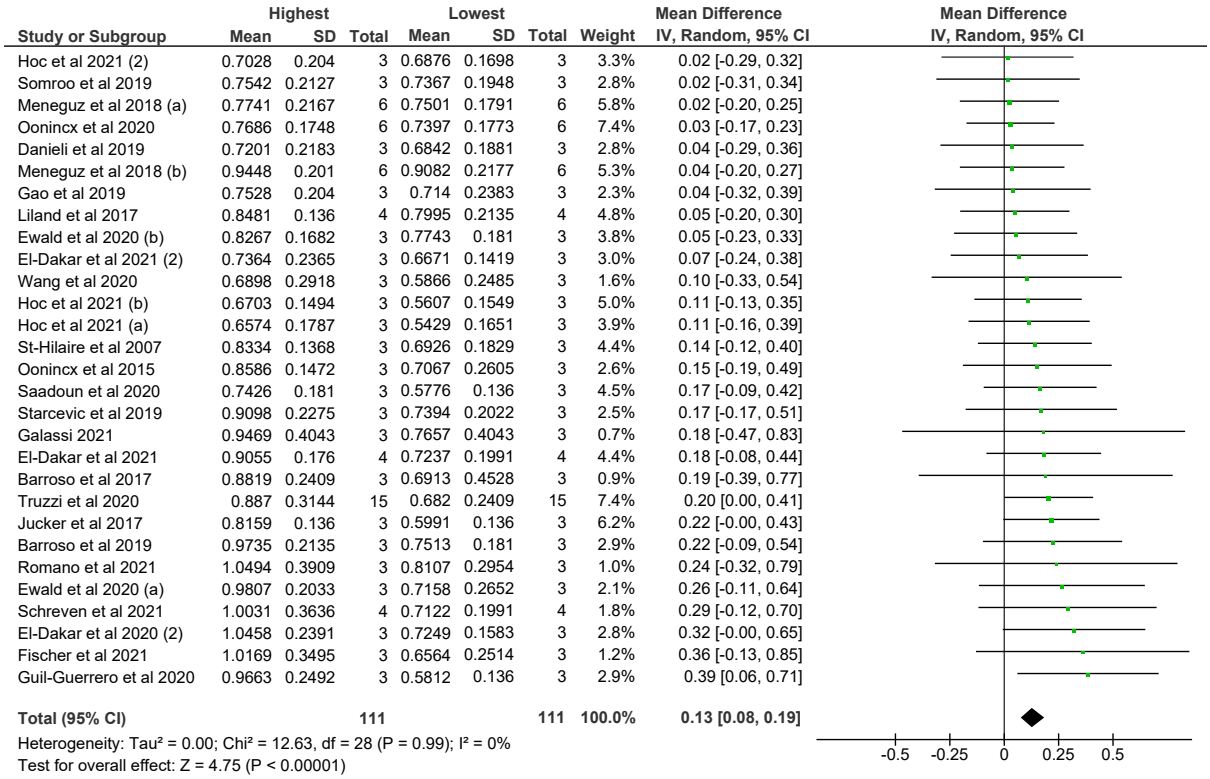


Figure 4.4: Software output of meta-analysis of palmitic acid concentration of black soldier fly larval fatty acid profile

4.6 Margaric acid

Margaric acid is a 17 carbon-chain saturated fatty acid (C17:0). It was the only odd-chain saturated fatty acid that was present in an adequate number of trials to be included in a meta-analysis. It is not naturally found in high amounts in either animal fats or plant oils, but

Stellenbosch University https://scholar.sun.ac.za

is sometimes found in palm oil and beef tallow (Kenar *et al.*, 2017). The absence of margaric acid in BSFL fatty acids reported by numerous trials indicates that it is not always present in BSFL fatty acid profiles. The highest observed concentration of margaric acid was 2.93% ± 0.32, which was found when the larvae were reared on a formulated wheat bran diet (Gao *et al.*, 2019), as seen in Table 4.2. Besides the one trial that reported a margaric acid concentration of 2.39%, all the other included trials reported concentrations lower than 1%. The largest identified effect size was found to be 0.50% [0.26,0.74] (Oonincx *et al.*, 2015).

The meta-analysis performed on the margaric acid concentration is illustrated by Figure 4.. The Chi^2 test indicated that the intervention effects were completely homogeneous (P = 1.00). This lack of heterogeneity was also suggested by the Tau^2 statistic and the I^2 statistic. The Z-test for the overall effect indicated that the rearing substrate did not have a significant effect on the margaric acid concentration of the larvae and that the observed difference in concentration was rather due to chance (P = 0.17).

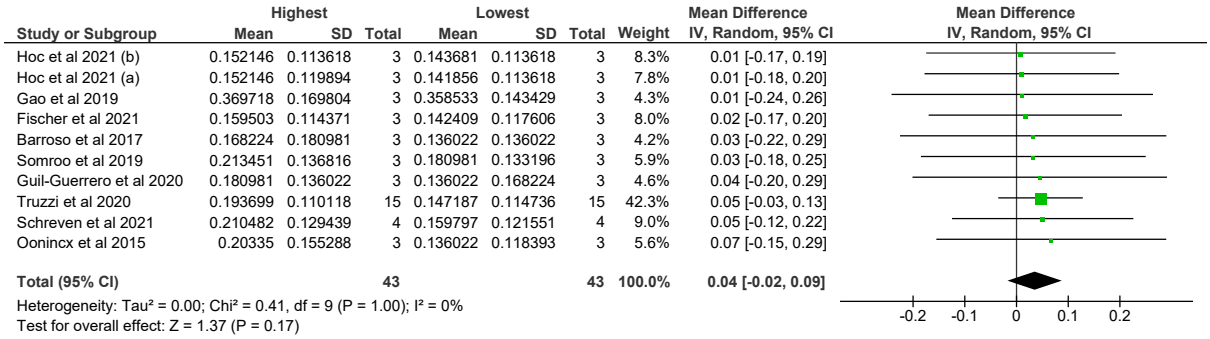


Figure 4.5: Software output of meta-analysis of margaric acid concentration of black soldier fly larval fatty acid profile

4.7 Stearic acid

Stearic acid is a long carbon chain saturated fatty acid (C18:0). It is the second most commonly found saturated fatty acid in nature. It is found in numerous plant oils and animal fats, including soybean and sunflower oil and beef tallow (Kenar *et al.*, 2017). Stearic acid was the fourth and final saturated fatty acid that was found to always be present in the BSFL fatty acid profile as it was observed by all 29 included trials. The lowest observed concentration of stearic acid was 0.09% ± 0.10 (Table 4.2) when the larvae were reared on fruit (Jucker *et al.*, 2017). The highest observed concentration was 21.40% ± 0.90 when the larvae were reared on coffee production by-product enriched with microalgae (Truzzi *et al.*, 2020). The largest identified difference in concentration was 16.70% (Truzzi *et al.*, 2020).

Figure 4. illustrates the meta-analysis results for the stearic acid concentration. The Chi^2 test indicated that there was no heterogeneity of importance (P = 0.07) and the I^2 statistic indicates that the heterogeneity that is present, is to a small enough extent as to not have an important impact on the meta-analysis results. The Tau^2 statistic also alluded to a small yet detectable level of heterogeneity. The Z-test for the overall effect indicated that the rearing substrate composition had a significant effect on the BSFL stearic acid concentration (P < 0.00001).

	Highest			Lowest				Mean Difference	Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	IV, Random, 95% CI
Gao et al 2019	0.30786	0.160162	3	0.295723	0.162657	3	2.9%	0.01 [-0.25, 0.27]	
Danieli et al 2019	0.255749	0.138034	3	0.233903	0.13174	3	3.8%	0.02 [-0.19, 0.24]	
Ewald et al 2020 (b)	0.378972	0.168224	3	0.356826	0.153732	3	2.9%	0.02 [-0.24, 0.28]	
Hoc et al 2021 (2)	0.294342	0.133196	3	0.266717	0.136816	3	3.8%	0.03 [-0.19, 0.24]	
Hoc et al 2021 (b)	0.292956	0.143429	3	0.264408	0.152226	3	3.3%	0.03 [-0.21, 0.27]	
Oonincx et al 2020	0.362486	0.159797	6	0.33324	0.139976	6	5.1%	0.03 [-0.14, 0.20]	
Somroo et al 2019	0.320802	0.149409	3	0.287339	0.174314	3	2.9%	0.03 [-0.23, 0.29]	
El-Dakar et al 2020 (2)	0.299145	0.129439	3	0.258934	0.12439	3	4.1%	0.04 [-0.16, 0.24]	
Wang et al 2020	0.305206	0.167466	3	0.261295	0.152226	3	3.0%	0.04 [-0.21, 0.30]	
Hoc et al 2021 (a)	0.276492	0.133196	3	0.224871	0.119894	3	4.1%	0.05 [-0.15, 0.25]	
Meneguz et al 2018 (a)	0.350496	0.14876	6	0.297849	0.125432	6	5.7%	0.05 [-0.10, 0.21]	
Romano et al 2021	0.367508	0.162657	3	0.306536	0.160162	3	2.9%	0.06 [-0.20, 0.32]	
Oonincx et al 2015	0.362486	0.133928	3	0.301179	0.17604	3	3.1%	0.06 [-0.19, 0.31]	
Meneguz et al 2018 (b)	0.36428	0.155887	6	0.301651	0.126976	6	5.5%	0.06 [-0.10, 0.22]	
Saadoun et al 2020	0.291562	0.118393	3	0.225793	0.13174	3	4.2%	0.07 [-0.13, 0.27]	
Galassi 2021	0.387485	0.21444	3	0.320802	0.21444	3	1.9%	0.07 [-0.28, 0.41]	
Liland et al 2017	0.429515	0.136022	4	0.345232	0.168224	4	3.9%	0.08 [-0.13, 0.30]	
El-Dakar et al 2021 (2)	0.344642	0.190342	3	0.255749	0.138034	3	2.8%	0.09 [-0.18, 0.35]	
El-Dakar et al 2021	0.384314	0.189206	4	0.286629	0.138034	4	3.5%	0.10 [-0.13, 0.33]	
Fischer et al 2021	0.365953	0.142794	3	0.26618	0.158378	3	3.2%	0.10 [-0.14, 0.34]	
Ewald et al 2020 (a)	0.42472	0.168224	3	0.280156	0.168224	3	2.8%	0.14 [-0.12, 0.41]	
Barroso et al 2019	0.512615	0.192588	3	0.356826	0.153732	3	2.6%	0.16 [-0.12, 0.43]	
Barroso et al 2017	0.461758	0.287339	3	0.294342	0.339289	3	0.9%	0.17 [-0.34, 0.67]	
Starcevic et al 2019	0.44729	0.192844	3	0.272776	0.113618	3	3.0%	0.17 [-0.08, 0.43]	
Schreven et al 2021	0.524617	0.24	4	0.318895	0.152146	4	2.6%	0.21 [-0.07, 0.48]	
St-Hilaire et al 2007	0.499523	0.136816	3	0.264408	0.136816	3	3.7%	0.24 [0.02, 0.45]	
Guil-Guerrero et al 2020	0.599053	0.192588	3	0.272776	0.192588	3	2.2%	0.33 [0.02, 0.63]	
Jucker et al 2017	0.466193	0.136022	3	0.133928	0.136022	3	3.8%	0.33 [0.11, 0.55]	
Truzzi et al 2020	0.968693	0.232128	15	0.457283	0.20335	15	5.7%	0.51 [0.36, 0.67]	
Total (95% CI)			**111**			**111**	**100.0%**	**0.12 [0.06, 0.17]**	

Heterogeneity: Tau² = 0.01; Chi² = 39.64, df = 28 (P = 0.07); I² = 29%
Test for overall effect: Z = 4.46 (P < 0.00001)

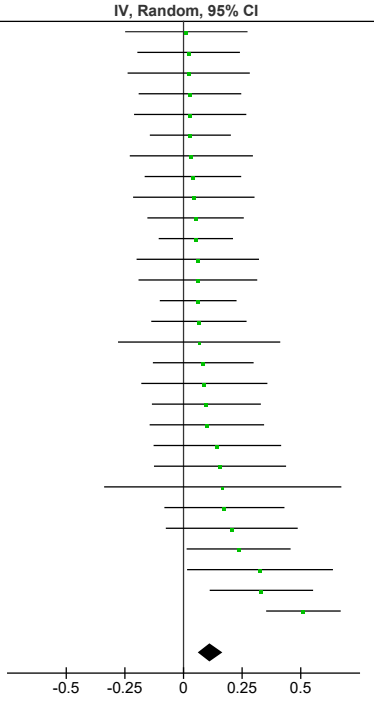


Figure 4.6: Software output of meta-analysis of stearic acid concentration of black soldier fly larval fatty acid profile

4.8 Arachidic acid

Arachidic acid is a long chain saturated fatty acid (C20:0). It is naturally found in small concentrations in canola, soybean and sunflower oil (Kenar *et al.*, 2017). It was reported to be present in the BSFL fatty acid profile by eight of the included trials. Therefore, it is possible for arachidic acid to be absent in the larvae. Table 4.2 shows that the highest observed concentration was 11.00% ± 0.30 when the larvae were reared on coffee production by-product, which itself wat reported to have a high concentration of arachidic acid (Truzzi *et al.*, 2020). Besides in the case of this one study, the arachidic acid concentration was never above 3%. The largest detected effect size was 9.80%, which was also found for the previously mentioned study.

Stellenbosch University https://scholar.sun.ac.za

The arachidic acid concentration meta-analysis can be seen in Figure 4.. The tests for heterogeneity also implied that there was a substantial amount of heterogeneity as the Chi^2 test exhibited a very low P-value ($P < 0.0001$) and the I^2 test statistic indicated 79% heterogeneity among the trials. The forest plot shows that the trial by Truzzi *et al.,* (2020) likely contributed to the heterogeneity to a large extent as its confidence interval does not overlap with most of the other trials. The substantial heterogeneity also impacted the average effect size as can be seen by the large confidence interval visualised in the forest plot. The Z-test for the overall effect indicated that the rearing substrate composition had no significant effect on the arachidic acid concentration ($P = 0.12$). A second meta-analysis was performed that excluded the one trial, to determine if there might be a significant effect if the heterogeneity were lower. The results of that meta-analysis can be seen in Appendix B (Figure B 1). Lower heterogeneity did not lead to a significant effect.

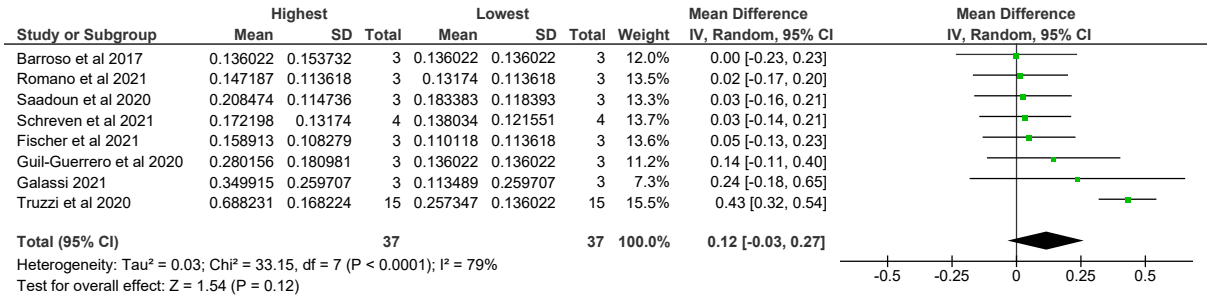


Figure 4.7: Software output of meta-analysis of arachidic acid concentration of black soldier fly larval fatty acid profile

4.9 Behenic acid

Behenic acid is a long chain saturated fatty acid (C22:0). It is naturally found in small quantities in canola and soybean oil (Kenar *et al.*, 2017). Only three trials reported its presence in the BSFL fatty acid profile, which implies that it is mostly absent in the larvae. The highest observed behenic acid concentration was 16.00% ± 0.40 when the larvae were reared on coffee silverskin (Truzzi *et al.*, 2020) as shown in Table 4.2. It should be noted that the other two trials reported behenic acid concentrations less than 1%. The previously mentioned trial also reported the largest difference in behenic acid concentration, which was 15%. In that trial the rearing substrate contained a noticeably higher amount of behenic acid compared to the rearing substrates used in the other two trials.

Stellenbosch University https://scholar.sun.ac.za

Figure 4. shows the results of the meta-analysis performed on the behenic acid concentration data. Considerable heterogeneity was detected (I^2 = 96%). This was affirmed by the Chi^2 test statistic. The Tau^2 statistic also displays a large variation. The heterogeneity could be completely attributed to one study, as its confidence interval did not overlap at all with the other two trials. The study's contribution can also be seen in the confidence interval of the average effect size. A second meta-analysis was performed where this study was excluded and can be seen in Appendix B (Figure B 2). The Z-test for the overall effect suggested that the rearing substrate composition did not have an effect on the behenic acid concentration of the larvae. Similar results were found for the second meta-analysis.

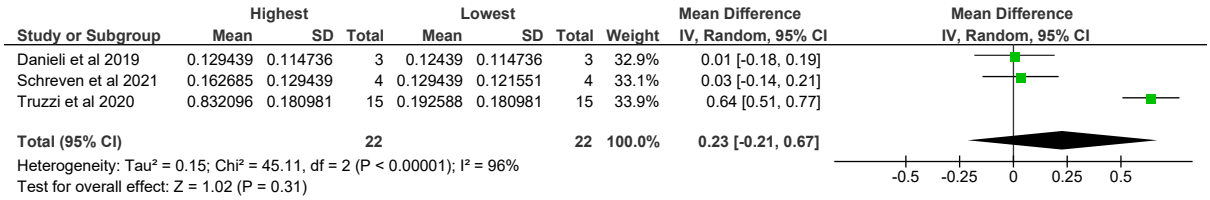


Figure 4.8: Software output of meta-analysis of behenic acid concentration of black soldier fly larval fatty acid profile

4.10 Total saturated fatty acids

The total amount of saturated fatty acids (SFAs) is generally reported by publications to indicate the level of saturation of the BSFL lipids. This concentration was however not reported for all 29 included trials. The meta-analysis of the total amount of SFA was only performed on the trials that did report it. In total 21 trials were included in this meta-analysis. As seen in Table 4.2, the lowest observed concentration of SFA was 28.11% ± 2.18 when the larvae were reared on crude olive oil cake, which is a by-product of the oil extraction process and is characterized by being high in unsaturated fatty acids (Starcevic *et al.*, 2019). The highest observed concentration of SFA was 86.89% ± 0.57 when the larvae were reared on a diet that consisted of 68% ground barley, 20% wheat bran and 12% dehydrated alfalfa (Danieli *et al.*, 2019). The largest identified effect size was 37.99% (Starcevic *et al.*, 2019).

Figure 4. reports the results of the meta-analysis performed on the total amount of saturated fatty acids. In regard to the test for heterogeneity, the Chi^2 test statistic had a P-value above 0.05 (P = 0.12), which implied that the intervention effects were homogeneous and could be compared by means of a meta-analysis. The I^2 statistic indicated 27% heterogeneity. This was however sufficiently low to not influence the meta-

analysis results. The Z-test for the overall effect indicated that the rearing substrate composition had a significant effect on the SFA concentration of the BSFL.

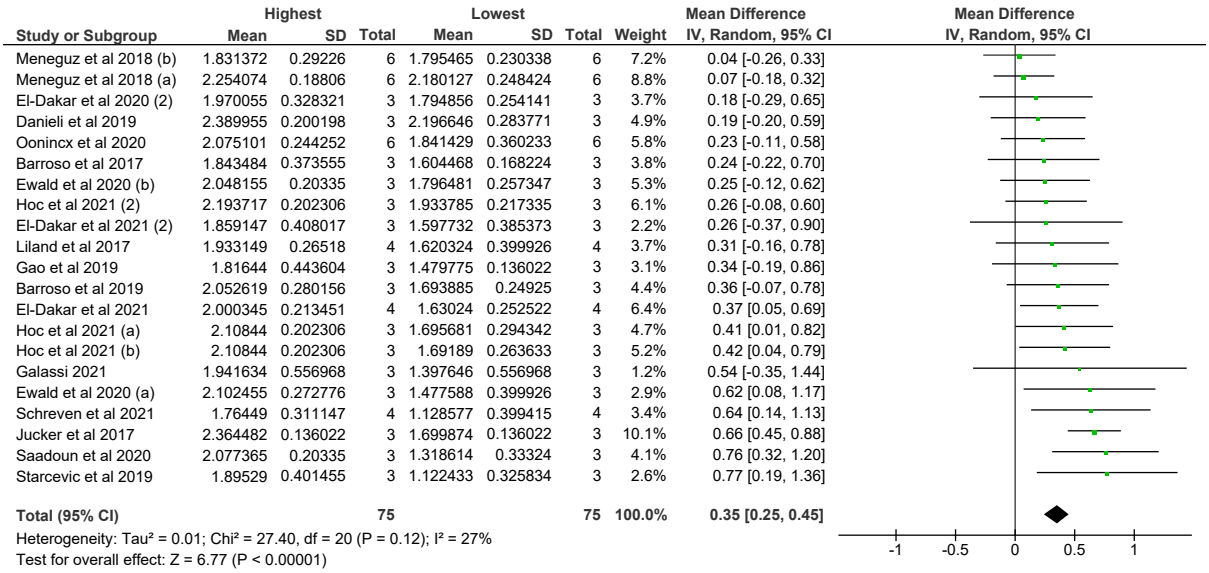


Figure 4.9: Software output of meta-analysis of the total saturated fatty acid concentration of black soldier fly larval fatty acid profile

4.11 Discussion

The meta-analyses were performed on the transformed data. Therefore, the scale of the average affect sizes could not be interpreted in the same way as the original data, which was presented in percentage of total fatty acids. The resulting average effect sizes from the different meta-analyses can however be compared to each other in terms of magnitude. Table 4.3 depicts the average effect sizes with their accompanying confidence intervals that were calculated by means of the meta-analyses.

Stellenbosch University https://scholar.sun.ac.za

Table 4.3: Average effect sizes (with confidence intervals) of the different fatty acids and total saturated fatty acid calculated by means of meta-analyses

Fatty acid	Average effect size (with confidence interval)	Z-test P-value	Chi^2 P-value	Tau^2	I^2
Capric acid	0.06 [0.02, 0.10]	0.006	1.00	0.00	0%
Lauric acid	0.44 [0.32, 0.55]	<0.00001	0.002	0.04	49%
Myristic acid	0.12 [0.08, 0.17]	<0.00001	0.98	0.00	0%
Palmitic acid	0.13 [0.08, 0.19]	<0.00001	0.99	0.00	0%
Margaric acid	0.04 [-0.02, 0.09]	0.17	1.00	0.00	0%
Stearic acid	0.12 [0.06, 0.17]	<0.00001	0.07	0.01	29%
Arachidic acid	0.12 [-0.03, 0.27]	0.12	<0.00001	0.03	79%
Behenic acid	0.23 [-0.21, 0.67]	0.31	<0.00001	0.15	96%
Total SFA	0.35 [0.25, 0.45]	<0.00001	0.12	0.01	27%

The average effect size refers to the influence that the rearing substrate composition had on the concentration of a specific saturated fatty acid in the BSFL lipids. Only three of the SFAs were not significantly affected by the rearing substrate composition. The largest effect was found for lauric acid. The second largest effect was found for the total amount of SFA. Lauric acid was most likely a major contributor to this. This would seem to indicate that it is possible to manipulate the lauric acid content and the level of saturation of the BSFL lipids though nutrition and feed formulation.

The table provides a relative scale to illustrate to which extent it is most likely possible to influence and thus change the concentrations of these different SFAs in the BSFL fatty acid profile. The table could be used as a guide to indicate which SFAs could be manipulated via rearing substrate formulation and to what extent.

4.12 Reference

Barroso, F.G., Sanchez-Muros, M.J., Rincon, M.A., Rodriguez-Rodriguez, M., Fabrikov, D., Morote, E. & Guil-Guerrero, J.L., 2019. Production of n-3-rich insects by bioaccumulation of fishery waste. J. Food Compos. Anal. 82, 103237. https://doi.org/10.1016/j.jfca.2019.103237

Barroso, F.G., Sánchez-Muros, M., Segura, M., Morote, E., Torres, A., Ramos, R. & Guil, J.L., 2017. Insects as food: enrichment of larvae of *Hermetia illucens* with omega 3 fatty acids by means of dietary modifications. J. Food Compos. Anal. 62, 8-13. https://doi.org/10.1016/j.jfca.2017.04.008

Stellenbosch University https://scholar.sun.ac.za

Danielie, P.P., Lussiana, C., Gasco, L., Amici, A. & Ronchi, B., 2019. The effect of diet formulation on the yield, proximate composition, and fatty acid profile of the black soldier fly (*Hermetia illucens* L.) prepupae intended for animal feed. Animals. 9, 178. doi:10.3390/ani9040178

El-Dakar, M.A., Ramzy, R.R. & Ji, H., 2021a. Influence of substrate inclusion of quail manure on the growth performance, body composition, fatty acid and amino acid profiles of black soldier fly larvae (*Hermetia illucens*). Sci. Total Environ. 772, 145528. https://doi.org/10.1016/j.scitotenv.2021.145528

El-Dakar, M.A., Ramzy, R.R., Ji, H. & Plath, M., 2020. Bioaccumulation of residual omega-3 fatty acids from industrial Schizochytrium microalgal waste using black soldier fly (*Hermetia illucens*) larvae. J. Clean. Prod. 268, 122288. https://doi.org/ 10.1016/j.jclepro.2020. 122288

El-Dakar, M.A., Ramzy, R.R., Wang, D. & Ji, H., 2021b. Sustainable management of Se-rich silkworm residuals by black soldier flies larvae to produce a high nutritional value and accumulate ω-3 PUFA. Waste Manag. 124, 72-81. https://doi.org/10.1016/ j.wasman.2021.01.040

Ewald, N., Vidakovic, A., Langeland, M., Kiessling, A., Sampels, S. & Lalander, C., 2020. Fatty acid composition of black soldier fly larvae (*Hermetia illucens*) - possibilities and limitations for modification through diet. Waste Manag. 102, 40-47 https://doi.org/10.1016/j.wasman.2019.10.014

Fischer, H., Roomano, N. & Sinha, A.K., 2021. Conversion of spent coffee and donuts by black soldier fly (*Hermetia illucens*) larvae into potential resources for animal and plant farming. Insects. 12, 332. https://doi.org/10.3390/insects12040332

Freeman, M.F. & Tukey, J.W. 1950. Transformations related to the angular and the square root. Ann. Mathematical Stat., 21, 607-611.

Galassi, G., Jucker, C., Parma, P., Lupi, D., Crovetto, G.M., Savoldelli, S. & Colombini, S., 2021. Impact of Agro-industrial byproducts on bioconversion, chemical composition, in vitro digestibility, and microbiota of the black soldier fly (Diptera: Stratiomyidae) larvae. J. Insect Sci. 21, 1-10. https://doi.org/10.1093/jisesa/ieaa148

Gao, Z., Wang, W., Lu, X., Zhu, F., Liu, W., Wang, X. & Lei, C., 2019. Bioconversion performance and life table of black soldier fly (*Hermetia illucens*) on fermented maize straw. J. Clean. Prod. 230, 974-980 https://doi.org/10.1016/j.jclepro.2019.05.074

Giannetto, A., Oliva, S., Ceccon Lanes, C.F., de Araújo Pedron, F., Savastano, D., Baviera, C., Parrino, V., Lo Paro, G., Spano, N.C., Cappello, T., Maisano, M., Mauceri, A. & Fasulo, S., 2020. *Hermetia illucens* (Diptera: Stratiomydae) larvae and prepupae: biomass production, fatty acid profile and expression of key genes involved in lipid metabolism. J. Biotechnol. 307, 44-54 https://doi.org/10.1016/j.jbiotec.2019.10.015

Stellenbosch University https://scholar.sun.ac.za

Hoc, B., Francis, F., Carpentier, J., Mostade, L., Blecker, C., Purcaro, G. & Caparros Megido, R., 2021a. ω3-enrichment of *Hermetia illucens* (L. 1758) prepupae from oilseed byproducts. J. Saudi Soc. Agric. Sci. 20, 155-163. https://doi.org/10.1016/j.jssas.2021.01.001

Hoc, B., Genva, M., Fauconnier, M-L., Lognay, G., Francis, F. & Megido, R.C., 2020. About lipid metabolism in *Hermetia illucens* (L. 1758): on the origin of fatty acids in prepupae. Sci. Rep. 10, 11916. https://doi.org/10.1038/s41598-020-68784-8

Hoc, B., Tomson, T., Malumba, P., Blecker, C., Jijakli, M.H., Purcaro, G., Francis, F. & Caparros Megido, R., 2021b. Production of rainbow trout (*Oncorhynchus mykiss*) using black soldier fly (*Hermetia illucens*) prepupae-based formulations with differentiated fatty acid profiles. Sci. Total Environ. 794, 148647. https://doi.org/10.1016/j.scitotenv.2021.148647

Jucker, C., Erba, D., Leonardi, M.G., Lupi, D. & Savoldelli, S., 2017. Assessment of vegetable and fruit substrates as potential rearing media for *Hermetia illucens* (Diptera: Stratiomyidae) larvae. Environ. Entomol. 46, 1415-1423. https://doi.org/10.1093/ee/nvx154

Kenar, J.A., Moser, B.R. & List, G.R., 2017. Chapter 2: Naturally occurring fatty acids: sources, chemistry, and uses. In: Fatty acids. 1st Edition. Ed: Ahmad, M.U., AOCS Press, London, United Kingdom. pp.24-71. https://doi.org/10.1016/B978-0-12-809521-8.00002-7

Liland, N.S., Biancarosa, I., Araujo, P, Biemans, D., Bruckner, C.G., Woogbø, R., Torstensen, B.E. & Lock, E.J., 2017. Modulation of nutrient composition of black soldier fly (*Hermetia illucens*) larvae by feeding seaweed-enriched media. PLoS One. 12. https://doi.org/10.1371/journal.pone.0183188

Meneguz, M., Schiavone, A., Gai, F., Dama, A., Lussiana, C., Renna, M. & Gasco, L., 2018. Effect of rearing substrate on growth performance, waste reduction efficiency and chemical composition of black soldier fly (*Hermetia illucens*) larvae. J. Sci. Food Agric. 98, 5776–5784. https://doi.org/10.1002/jsfa.9127

Oonincx, D.G.A.B., van Broekhoven, S., van Huis, A. & van Loon, J.J.A., 2015. Feed conversion, survival and development, and composition of four insect species on diets composed of food by-products. PLoS One. 10. https://doi.org/10.1371/journal.pone.0144601

Oonincx, D.G.A.B., Laurent, S., Veenenbos, M.E. & van Loon, J.J.A., 2020. Dietary enrichment of edible insects with omega 3 fatty acids. Iinsect Sci. 27, 500-509. https://doi.org/10.1111/1744-7917.12669

Stellenbosch University https://scholar.sun.ac.za

Romano, N., Fischer, H., Kumar, V., Francis, S.A. & Sinha, A.K., 2021. Productivity, conversion ability, and biochemical composition of black soldier fly (*Hermetia illucens*) larvae fed with sweet potato, spent coffee or dough. Int. J. Trop. Insect Sci. https://doi.org/10.1007/s42690-021-00532-5

Saadoun, J.H., Montevecchi, G., Zanasi, L., Bortolini, S., Macavei, L.I., Masino, F., Maistrello, L. & Antonelli, A., 2020. Lipid profile and growth of black soldier flies (*Hermetia illucens*, Stratiomyidae) reared on by-products from different food chains. J. Sci. Food Agric. 100, 3648-3657. https://doi.org/10.1002/jsfa.10397

Schreven, S.J.J., Yener, S., van Valenberg, H.J.F., Dicke, M. & van Loon, J.J.A., 2021. Life on a piece of cake: performance and fatty acid profiles of black soldier fly larvae fed oilseed by-products. J. Insects as Food Feed. 7, 35-49. https://doi.org/ 10.3920/JIFF2020.0004

Somroo, A.A., ur Rehman, K., Zheng, L., Cai, M., Xiao, X., Hu, S., Mathys, A., Gold, M., Yu, Z. & Zhang, J., 2019. Influence of *Lactobacillus buchneri* on soybean curd residue co-conversion by black soldier fly larvae (*Hermetia illucens*) for food and feedstock production. Waste Manag. 86, 114-122. https://doi.org/10.1016/j.wasman.2019.01.022

St-Hilaire, S., Cranfill, K., McGuire, M.A., Mosley, E.E., Tomberlin, J.K., Newton, L., Sealey, W., Sheppard, C. & Irving, S., 2007. Fish offal recycling by black soldier fly produces a foodstuff high in omega-3 fatty acids. J. World Aquac. Soc. 38, 197-202. https://doi.org/10.1111/j.1749-7345.2007.00101.x

Starcevic, K., Lozica, L., Gavrilovic, A., Heruc, Z. & Masek, T., 2019. Fatty acid plasticity of black soldier fly (*Hermetia illucens*) larvae reared on alternative feeding media: crude olive cake and processed animal protein. J. Anim. Feed Sci. 28, 374-382. https://doi.org/10.22358/jafs/114434/2019

Truzzi, C., Giorgini, E., Annibaldi, A., Antonucci, M., Illuminati, S., Scarponi, G., Riolo, P., Isidoro, N., Conti, C., Zarantoniello, M., Cipriani, R. & Olivotto, I., 2020. Fatty acids profile of black soldier fly (*Hermetia illucens*): influence of feeding substrate based on coffee-waste silverskin enriched with microalgae. Anim. Feed Sci. Technol. 259, 114309. https://doi.org/10.1016/j.anifeedsci.2019.114309

Wang, S.Y., Wu, L., Li, B. & Zhang, D., 2020. Reproductive potential and nutritional composition of *Hermetia illucens* (Diptera: Stratiomyidae) prepupae reared on different organic wastes. J. Econ. Entomol. 113, 527-537. https://doi.org/10.1093/jee/toz296

Stellenbosch University https://scholar.sun.ac.za

CHAPTER 5

Meta-analyses of monounsaturated fatty acids

Monounsaturated fatty acids (MUFAs), as the name suggests, are fatty acids that have a single double bond. The black soldier fly larval (BSFL) fatty acid profile generally contains less MUFAs compared to the saturated fatty acid component. They are however present and some of them play an important role in BSFL metabolism and other biological functions. From a nutritional point of view, MUFAs contribute to a healthy diet for both humans and animals, and they are therefore of interest. Monounsaturated fatty acids can be biosynthesized through metabolic pathways that involve desaturase and chain elongation enzyme reactions (Kenar *et al.*, 2017). Due to the biosynthesis process, some MUFAs might be present in the BSFL fatty acid profile, even if they were absent in the rearing substrate.

As with the meta-analyses performed for the saturated fatty acids, data from 29 trials that originated from 26 studies were used to perform meta-analyses (**Error! Reference source not found.** in Appendix A). In total 17 MUFAs were identified to be present in the BSFL fatty acid profile, as illustrated in Table 5.. Only one MUFA, oleic acid, was found to be consistently present in the larval fatty acid profile. Seven of the MUFAs were reported by a sufficient number of trials for meta-analyses to be performed. A meta-analysis was also performed on data regarding the total amount of MUFA identified in the larval fatty acid profile.

Stellenbosch University https://scholar.sun.ac.za

Table 5.1: List of monounsaturated fatty acids identified as present in the black soldier fly larval fatty acid profile

Common name	Preferred IUPAC name	Omega nomenclature	Inclusion in meta-analysis
Tridecenoic acid	9-cis-tridecenoic acid	C13:1 cis-9	No
Myristoleic acid	9-cis-tetradecenoic acid	C14:1 n-5	Yes
Pentadecenoic acid	10-cis-pentadecenoic acid	C15:1 n-5	Yes
Palmitoleic acid	9-cis-hexadecenoic acid	C16:1 cis-9	Yes
Hexadecenoic acid	9-trans-hexadecenoic acid	C16: trans-9	No
Heptadecenoic acid	10-cis-heptadecenoic acid	C17:1 n-7	Yes
Oleic acid	9-cis-octadecenoic acid	C18:1 cis-9	Yes
Elaidic acid	9-trans-octadecenoic acid	C18:1 trans-9	No
Vaccenic acid	11-trans-octadecenoic acid	C18:1 trans-11	Yes
Nonadecenoic acid	10-cis-nonadecenoic acid	C19:1 cis-10	No
Nonadecenoic acid	11-trans-nonadecenoic acid	C19:1 trans-10	No
Gondoic acid	11-cis-eicosenoic acid	C20:1 cis-11	Yes
Heneicosenoic acid	12-cis-heneicosenoic acid	C21:1 cis-12	No
Cetoleic acid	11-cis-docosenoic acid	C22:1 cis-11	No
Erucic acid	13-cis-docosenoic acid	C22:1 cis-13	No
Docosenoic acid	13-trans-docosenoic acid	C22:1 trans-13	No
Nervonic acid	15-cis-tetracosenoic acid	C24:1 cis-15	No

5.1 Summary statistics

Table 5.5 illustrates the consolidated data regarding the lowest and highest observed concentrations of the individual monounsaturated fatty acids (MUFAs) as well as the largest difference in concentration identified by a single study. Oleic acid was the only MUFA that was consistently present in the BSFL fatty acid profile. Therefore, its lowest concentration is indicated in the table. For all the other MUFAs the lowest concentration was noted as zero to

Stellenbosch University https://scholar.sun.ac.za

indicated that they were occasionally absent in the fatty acid profile. Oleic acid was found to have the highest observed concentration with 54.10% (as % of total fatty acids), which was almost 30% higher than the highest observed concentrations of any of the other MUFAs (Starcevic *et al.*, 2019). The concentration of the total amount of MUFA in the BSFL fatty acid profile varied from 7.20% (Hoc *et al.*, 2021a) to 63.37% (Schreven *et al.*, 2021). The largest differences in concentration from a single trial was also reported for the oleic acid concentration and the total amount of MUFA (Starcevic *et al.*, 2019). As both reported differences are from the same study, it is safe to assume that the oleic acid was responsible for the change in MUFA concentration.

Table 5.5: Summary statistics regarding concentrations of individual monounsaturated fatty acids (MUFAs) in black soldier fly larval fatty acid profile (in % of total fatty acids)

Fatty acid	**Lower concentration limit (mean ± SD)**	**Higher concentration limit (mean ± SD)**	**Largest difference in concentration**
Myristoleic acid	0*	1.39 ± 0.08	0.91 [0.82, 1.00]
(C14:1 n-5)		(El-Dakar *et al.*, 2021a)	(El-Dakar *et al.*, 2021a)
Pentadecenoic acid	0*	0.50 ± 0.05	0.13 [-0.12, 0.38]
(C15:1 n-5)		(Somroo *et al.*, 2019)	(Hoc *et al.*, 2021a)
Palmitoleic acid	0*	15.00 ± 0.86	11.50 [10.93, 12.07]
(C16:1 cis-9)		(El-Dakar *et al.*, 2021a)	(Ewald *et al.*, 2020)
Heptadecenoic acid	0*	1.30 ± 0.173	0.50 [0.30, 0.70]
(C17:1 n-7)		(Somroo *et al.*, 2019)	(Somroo *et al.*, 2019)
Oleic acid	4.75 ± 0.21	54.12 ± 1.82	39.42 [36.76, 42.08]
(C18:1 cis-9)	(Danieli *et al.*, 2019)	(Starcevic *et al.*, 2019)	(Starcevic *et al.*, 2019)
Vaccenic acid	0*	2.50 ± 0.30	2.10 [0.49, 0.99]
(C18:1 trans-11)		(Truzzi *et al.*, 2020)	(Ewald *et al.*, 2020)
Gondoic acid	0*	2.05 ± 0.24	1.99 [1.75, 2.23]
(C20:1 cis-11)		(Schreven *et al.*, 2021)	(Schreven *et al.*, 2021)
Total MUFA	7.20 ± 0.16	63.37 ± 3.04	42.44 [39.75, 45.13]
		(Schreven *et al.*, 2021)	(Starcevic *et al.*, 2019)

* Fatty acid was absent in black soldier fly larvae in some included trials.
All values presented as % of total fatty acids.
SD – standard deviation.
CI – confidence interval.

Stellenbosch University https://scholar.sun.ac.za

The fatty acid data collected from the 29 trials was in the form of the concentration of each individual in respect to the BSFL fatty acid profile (mean ± standard deviation) and the analysis sample sizes. Some of the studies reported the results as mean ± standard error. In those cases the standard deviation were calculated using Equation 5.10.

Equation 5.10: Standard deviation equation

$$Standard\ deviation = standard\ error\ \times \sqrt{sample\ size}$$

Preliminary meta-analyses were performed with the collected data, but it was found that the data was too heterogeneous for accurate comparisons to be made and that the results could lead to misleading conclusions. The data was therefore transformed using the Freeman-Tukey double arcsine transformation. This was done to increase the homogeneity so as to allow for accurate comparisons. The transformation equation (Equation 5.11) can be seen below, y_i is the original data point and $\hat{y}_i$ is the transformed data point:

Equation 5.11: Freeman-Tukey double arcsine transformation

$$\hat{y}_i\ = \arcsin\sqrt{y_i/(100+1)} + \ arcsin\sqrt{(y_i+1)/(100+1)}$$

The original scale of the collected data was percentage of total fatty acids. The transformed data and consequent meta-analysis results were on the scale of the transformation. The results were not back-transformed, as this has a high risk of producing misleading results.

Each meta-analysis consisted of several steps. A weighted intervention effect size, also known as a difference in means, was calculated for each trial. These intervention effects were then used to calculate the average effect size, reported as a mean with a confidence interval. The differences in means and the average effect size is then plotted on a forest plot to visualise the overlap of confidence intervals, which indicate the level of homogeneity among the intervention effects. The Chi^2 test was also performed to calculate it there was heterogeneity among the intervention effects. The null hypothesis for the test is that there was no heterogeneity and the alternative hypothesis was that there was heterogeneity that could potentially influence the results of the meta-analysis. The test is accompanied by the Tau^2 statistic and the I^2 statistic. The Tau^2 statistic is an indication of the variation among the intervention effects and the I^2 statistic report the percentage of heterogeneity. Based on the percentage of heterogeneity, a decision is made as to if the data is homogeneous enough to

perform a meta-analysis. The Z-test for the overall effect was then performed. The null hypothesis for the Z-test was that the rearing substrate composition had no significant effect on the concentration of the individual MUFA in the BSFL fatty acid profile. The alternative hypothesis was consequently that the rearing substrate composition did have a significant effect on the concentration of the individual fatty acid in the BSFL fatty acid profile. The significance was set at 5% for both the Z-test and the Chi^2 test (P = 0.05). This protocol was followed for each of the seven MUFAs as well as the total amount of MUFA in the BSFL fatty acid profile.

5.2 Myristoleic acid

Myristoleic acid is a MUFA that consist of a 14 carbon-chain with one double bond (C14:1 n-5). It is not frequently found as a component of natural fats and oils. Ten of the trials reported its presence in the BSFL fatty acid profiles. The highest observed concentration was 1.39% when the larvae were reared on quail manure (El-Dakar *et al.*, 2021a). The study did not report the presence of myristoleic acid in the substrate, but it did have a high concentration of MUFA in the form of oleic acid. The largest reported difference in concentration was 0.91% (El-Dakar *et al.*, 2021a).

The meta-analysis of the myristoleic acid data accompanied by a forest plot can be seen in Figure 5.. The Chi^2 test indicated that the intervention effects were homogenous (P = 1.00). Both the Tau^2 statistic and the I^2 statistic also indicated that there was no heterogeneity of importance to be considered before the meta-analysis was performed. The Z-test for the overall effect suggest that the rearing substrate composition did not have an effect on the myristoleic acid concentration in the BSFL fatty acid profile.

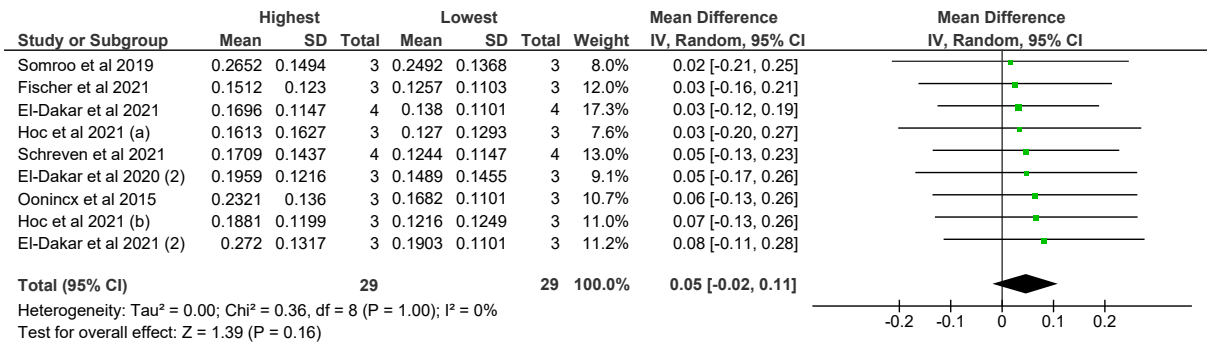


Figure 5.1: Software output of meta-analysis of myristoleic acid concentration of black soldier fly larval fatty acid profile

Stellenbosch University https://scholar.sun.ac.za

5.3 Pentadecenoic acid

Pentadecenoic acid is MUFA that consists of a 15 carbon-chain with a single double bond (C15:1 n-5). Pentadecenoic acid is uncommon in natural sources, and it was only detected in the BSFL fatty acid profile by three of the trials. The highest observed pentadecenoic acid concentration was 0.5% when the larvae were reared on soybean curd residue (Somroo *et al.*, 2019). The highest observed concentration for this MUFA was lower than any of the other detected MUFAs. The largest difference in concentration reported by a single trial was 0.13% (Hoc *et al.*, 2021a).

Figure 5. illustrates the meta-analysis performed with the pentadecenoic acid data, with the forest plot depicted. The Chi^2 test indicated that there was no heterogeneity (P = 0.98). The Tau^2 statistic indicated that there was no variation. This was also the case with the I^2 statistic, which indicated that there was 0% heterogeneity. The Z-test for the overall effect suggested that the rearing substrate composition had no significant effect on pentadecenoic acid concentration of the BSFL.

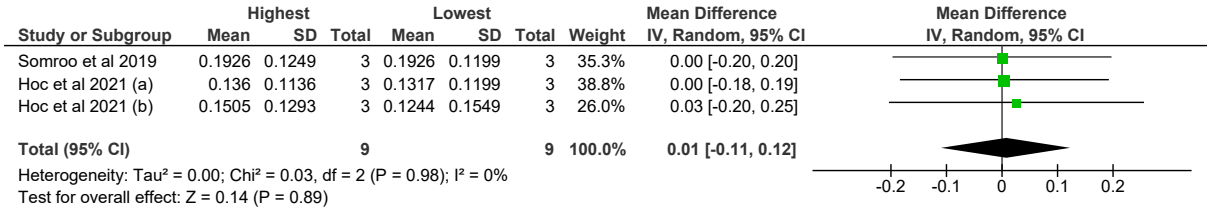


Figure 5.2: Software output of meta-analysis of pentadecenoic acid concentration of black soldier fly larval fatty acid profile

5.4 Palmitoleic acid

Palmitoleic acid is a MUFA that consists of a 16 carbon-chain with one double bond (C16:1 cis-9). It is found in small quantities in plant oils such as olive oil and it can be biosynthesized through the action of desaturase on other fatty acids such as palmitic acid (C16:0) (Kenar *et al.*, 2017). Most of the trials reported the detection of palmitoleic acid in the BSFL fatty acid profile. The highest observed palmitoleic acid concentration was 15.00% when the larvae were reared on quail manure, which was high in oleic acid (El-Dakar *et al.*, 2021a). The largest reported difference in concentration was 11.50% (Ewald *et al.*, 2020).

The meta-analysis result, the forest plot and the statistical output are shown in Figure 5.. The Chi^2 test indicated that there was no heterogeneity among the intervention effects that could influence the results (P = 0.51). The Tau^2 statistic and the I^2 statistic also indicated that there was no heterogeneity. The Z-test for the overall effect suggested that the rearing substrate composition had a significant effect on the palmitoleic acid concentration of the BSFL as it had a very low P-value (P < 0.00001).

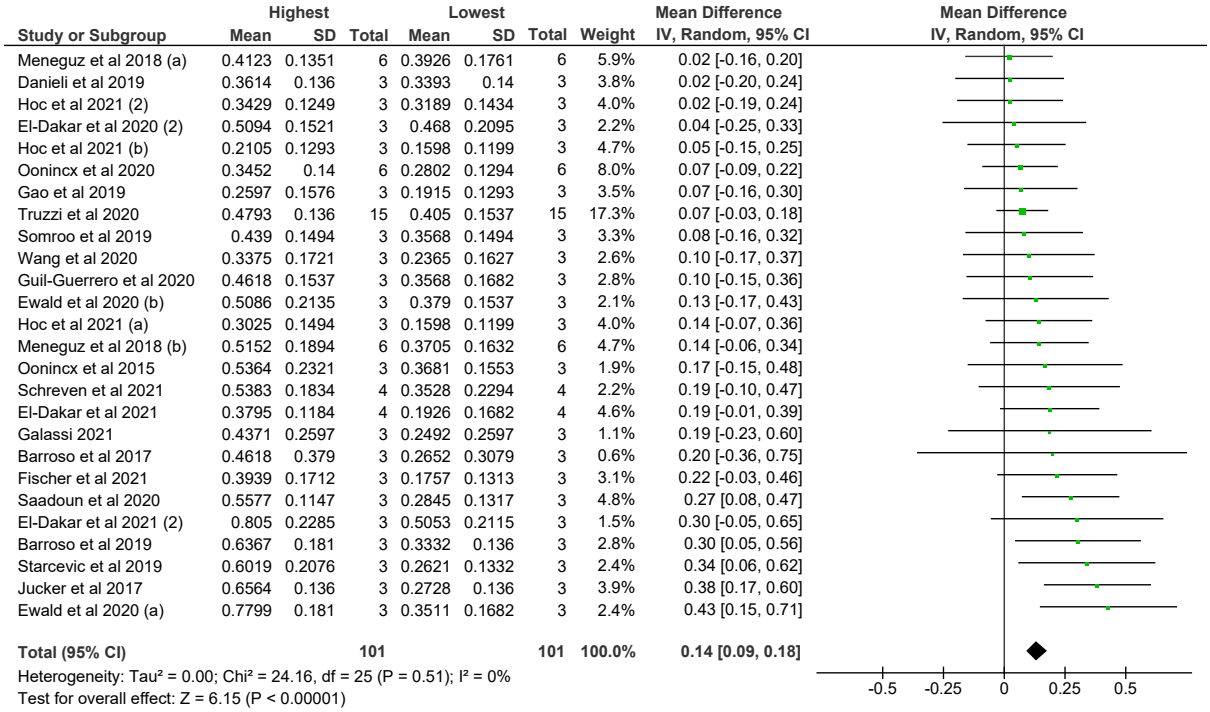


Figure 5.3: Software output of meta-analysis of palmitoleic acid concentration of black soldier fly larval fatty acid profile

5.5 Heptadecenoic acid

Heptadecenoic acid is a MUFA that consists of a 17 carbon-chain with one double bond (C17:1 n-7). It was the only other odd chain MUFA, besides pentadecenoic acid, that was detected by a sufficient number of trials for a meta-analysis to be performed. Heptadecenoic acid is uncommon in natural oils and fats. It is however found in small quantities in ruminant milk (Alves *et al.*, 2006). Three of the trials reported heptadecenoic acid in their analysed BSFL fatty acid profiles. The highest observed concentration was 1.30% when the larvae were reared on a formulated artificial feed (Somroo *et al.*, 2019). The same trial reported the largest difference in concentration, which was 0.5%.

Figure 5. illustrates the meta-analysis results with the accompanying forest plot and the statistical output. The Chi^2 test indicated that there was no heterogeneity among the intervention effects. This was also the case with the Tau^2 statistic and the I^2 statistic. The Z-test for the overall effect indicated that the rearing substrate composition did not have an effect on the heptadecenoic acid concentration of the larvae.

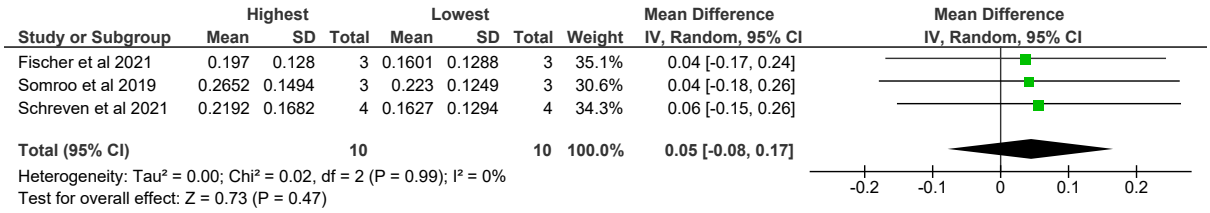


Figure 5.4: Software output of meta-analysis of heptadecenoic acid concentration of black soldier fly larval fatty acid profile

5.6 Oleic acid

Oleic acid is a fatty acid that consists of an 18 carbon-chain with one double bond (C18:1 cis-9). It is the most prevalent MUFA in natural oils and fats and can be found in high concentrations in plant oils such as olive and canola oil (Kenar *et al.*, 2017). It can also be present in pork lard and beef tallow. Oleic acid was the only MUFA that was reported as part of the BSFL fatty acid profile by all 29 trials. The lowest observed oleic acid concentration was 4.75% when the larvae were reared on a formulated feed consisting of 68% ground barley, 20% wheat bran and 12% dehydrated alfalfa (Danieli *et al.*, 2019). The highest observed concentration was 54.12% when the larvae were reared on crude olive cake, which is a by-product of olive oil production (Starcevic *et al.*, 2019). The crude olive cake was itself noticeably high in oleic acid (73.31%). The largest reported difference in concentration was 39.42%, which was the largest reported difference of any of the MUFAs reported in this study (Starcevic *et al.*, 2019).

The meta-analysis performed on the oleic acid data and the resulting statistics and forest plot is shown in Figure 5.. The Chi^2 test indicated that there was no noticeable heterogeneity among the intervention effects. The Tau^2 statistic also indicated that there was no variation. The I^2 statistic however indicated that there was 14% heterogeneity. The forest plot also illustrates that there was a slight deviation of overlap of the intervention effect confidence intervals for some of the trials. This percentage of heterogeneity was however low enough to as to not significantly influence the results of the meta-analysis. The Z-test for the overall

effect indicated that the rearing substrate composition had a significant effect on the oleic acid concentration of the BSFL as the P-value was very small (P < 0.00001).

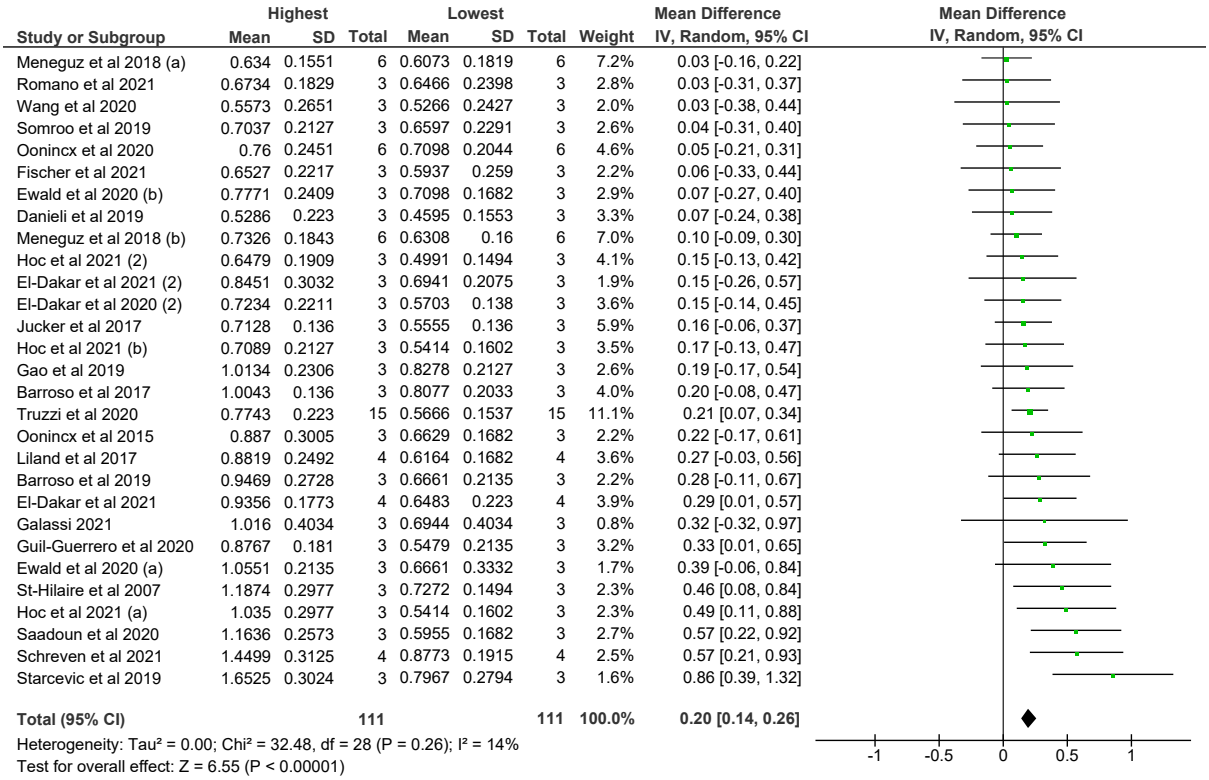


Figure 5.5: Software output of meta-analysis of oleic acid concentration of black soldier fly larval fatty acid profile

5.7 Vaccenic acid

Vaccenic acid is MUFA that consists of an 18 carbon-chain with one double bond (C18:1 trans-11). The difference between oleic acid and vaccenic acid is that vaccenic acid is a trans fatty acid. It is a naturally occurring trans fatty acid, found in ruminant milk, and was the only trans MUFA that was reported by a sufficient number of trials for a meta-analysis to be performed. Vaccenic acid was found to be present in the BSFL fatty acid profile by 13 of the trials. The highest observed concentration was 2.50% when the larvae were reared on coffee silverskin, a by-product of the coffee industry and which itself contained vaccenic acid (Truzzi *et al.*, 2020). The largest reported difference in vaccenic acid concentration was 2.10% (Ewald *et al.*, 2020).

Figure 5. shows the results of the meta-analysis performed on the vaccenic acid data. The Chi^2 test indicated that there was no heterogeneity among the intervention effect. The Tau^2 statistic and the I^2 statistic both confirmed the homogeneity. The Z-test for the overall effect

Stellenbosch University https://scholar.sun.ac.za

indicated that the rearing substrate composition had a significant effect on the vaccenic acid concentration of the larvae (P = 0.005).

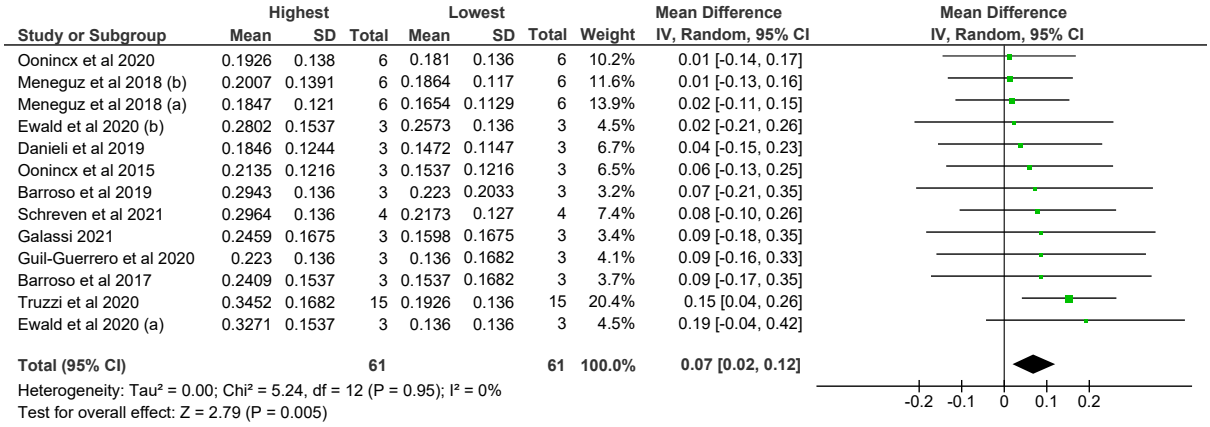


Figure 5.6: Software output of meta-analysis of vaccenic acid concentration of black soldier fly larval fatty acid profile

5.8 Gondoic acid

Gondoic acid is a MUFA that consists of a 20 carbon-chain with one double bond (C20:1 cis-11). It if found in small quantities in rapeseed and canola oil (Kenar *et al.*, 2017). Seven of the trials detected gondoic acid in the BSFL fatty acid profile. The highest reported gondoic acid concentration was 2.05% when the larvae were reared on a substrate that consisted of 50% chicken feed and 50% camelina pressed cake (Schreven *et al.*, 2021). The camelina pressed cake reportedly contained 7.81% gondoic acid (as % of total fatty acids). The largest reported difference in concentration was 1.99% and was reported by the same study.

The meta-analysis performed on the gondoic acid concentration data is reported in Figure 5.. The Chi^2 test indicated that there was no heterogeneity among the intervention effects. Both the Tau^2 statistic and the I^2 statistic indicated that there was no measure of heterogeneity. The Z-test for the overall effect suggested that the rearing substrate composition had no significant effect on the gondoic acid concentration of the larvae.

Stellenbosch University https://scholar.sun.ac.za

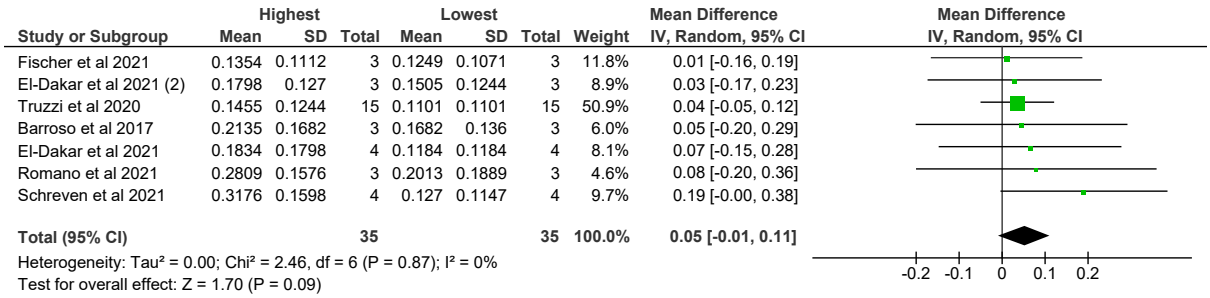


Figure 5.7: Software output of meta-analysis of gondoic acid concentration of black soldier fly larval fatty acid profile

5.9 Total monounsaturated fatty acids

Fatty acids are divided into two broad groups, saturated fatty acids (SFAs) and unsaturated fatty acids (UFAs), which indicates the presence or absence of double bonds. Unsaturated fatty acids are then further divided into monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), which indicate if there is one or more double bonds. The MUFA content of a fatty acid profile assists therefore in reporting the structural and functional properties of the fatty acid profile. The MUFA content of the BSFL was reported by 20 of the trials. The lowest observed MUFA concentration was 7.20% when the larvae were reared on chicken feed (Hoc *et al.*, 2021a). The diet mostly consisted of PUFAs and the BSFL fatty acid profile mostly consisted of SFAs. The highest reported MUFA concentration was 63.37% when the larvae were reared on *crambe* pressed cake, which was reportedly high in both mono- and polyunsaturated fatty acids (Schreven *et al.*, 2021).

Figure 5.11 shows the results of the meta-analysis performed on the MUFA data. The Chi^2 test indicated that there was heterogeneity among the intervention effects that could influence the meta-analysis results (P = 0.04). The Tau^2 statistic also indicated that there was some variation. The I^2 statistic indicated that there was 38% heterogeneity. This percentage of heterogeneity was still sufficiently low for it not to affect the meta-analysis. The Z-test for the overall effect suggested that the rearing substrate composition had a significant effect on the MUFA concentration of the BSFL fatty acid profile.

Stellenbosch University https://scholar.sun.ac.za

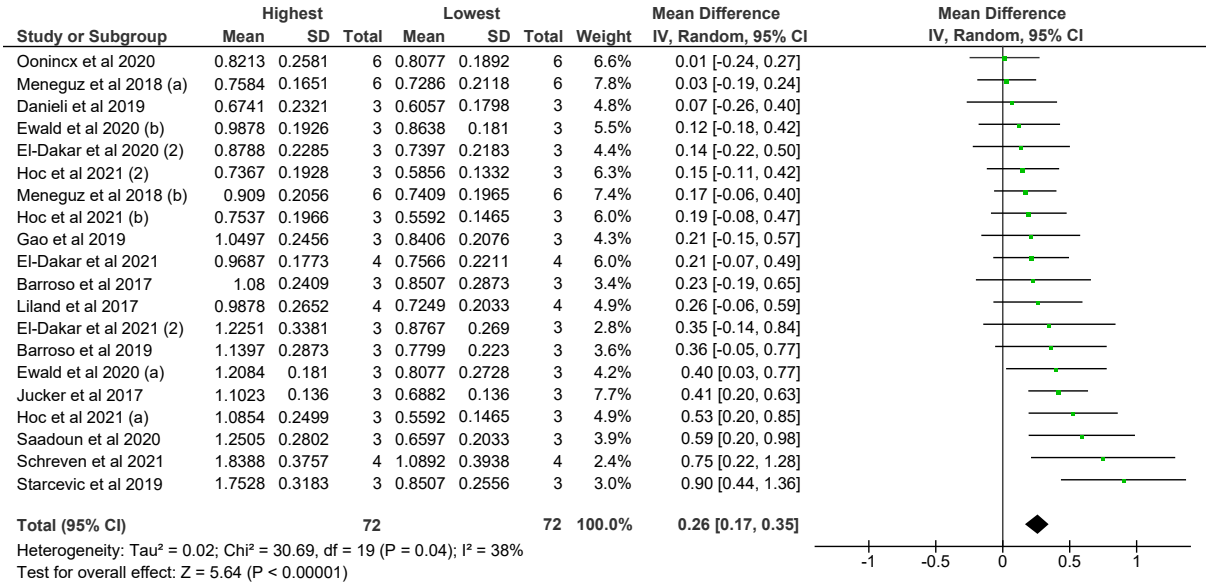


Figure 5.11: Software output of meta-analysis of the total concentration of monounsaturated fatty acids of black soldier fly larval fatty acid profile

5.10 Discussion

The meta-analyses performed on the concentrations of the individual monounsaturated fatty acids (MUFAs) and the total amount of MUFA calculated the heterogeneity among the observed intervention effects as well as the significance of the observed effect. Additionally, it calculated the average intervention effect sizes as means with accompanying confidence intervals. The meta-analyses results are illustrated in Table 5.. Back-transformations were not performed on the results as literature suggested that back-transformation of the Freeman-Tukey double arcsine transformation could bare misleading results. Therefore, the results were interpreted on the transformed scale.

Stellenbosch University https://scholar.sun.ac.za

Table 5.3: Average effect size (with confidence intervals) of rearing substrate composition on the different individual monounsaturated fatty acid concentrations calculated by the meta-analyses

Fatty acid	Average effect size (with CI)	Z-test P-value	Chi^2 P-value	Tau^2	I^2
Myristoleic acid	0.05 [-0.02, 0.11]	0.16	1.00	0.00	0%
Pentadecenoic acid	0.01 [-0.11, 0.12]	0.89	0.98	0.00	0%
Palmitoleic acid	0.14 [0.09, 0.18]	<0.00001	0.51	0.00	0%
Heptadecenoic acid	0.05 [-0.08, 0.17]	0.47	0.99	0.00	0%
Oleic acid	0.20 [0.14, 0.26	<0.00001	0.26	0.00	14%
Vaccenic acid	0.07 [0.02, 0.12]	0.005	0.95	0.00	0%
Gondoic acid	0.05 [-0.01, 0.11]	0.09	0.87	0.00	0%
Total MUFA	0.26 [0.17, 0.35]	<0.00001	0.04	0.02	38%

The rearing substrate composition had a significant effect on the palmitoleic, oleic and vaccenic acid concentrations of the larvae. The largest effect was seen on the oleic acid concentration, which was also the only MUFA that were reported as part of the BSFL fatty acid profile by all 29 trials. The total amount of MUFA was also significantly affected by the rearing substrate composition. The concentration of the other MUFAs, which included myristoleic, pentadecenoic, heptadecenoic and gondoic acid, were not affected by the rearing substrate composition. This leads to the conclusion that the larvae biosynthesize these fatty acids by converting other fatty acids, obtained through their diet, in the amounts needed for proper biological function independent of the rearing substrate composition.

These results suggest that it is possible to modify the BSFL fatty acid profile to an extent through the formulation of the rearing substrate, specifically, in regards to the palmitoleic, oleic and vaccenic acid and the total amount of MUFA.

5.11 References

Alves, S.P., Marcelino, C., Portugal, P.V. & Bessa, R.J.B., 2006. Short communication: The nature of heptadecenoic acid in ruminant fats. J. Dairy Sci. 89, 170-173 https://doi.org/10.3168/jds.S0022-0302(06)72081-1

Stellenbosch University https://scholar.sun.ac.za

Barroso, F.G., Sanchez-Muros, M.J., Rincon, M.A., Rodriguez-Rodriguez, M., Fabrikov, D., Morote, E. & Guil-Guerrero, J.L., 2019. Production of n-3-rich insects by bioaccumulation of fishery waste. J. Food Compos. Anal. 82, 103237. https://doi.org/10.1016/j.jfca.2019.103237

Barroso, F.G., Sánchez-Muros, M., Segura, M., Morote, E., Torres, A., Ramos, R. & Guil, J.L., 2017. Insects as food: enrichment of larvae of *Hermetia illucens* with omega 3 fatty acids by means of dietary modifications. J. Food Compos. Anal. 62, 8-13. https://doi.org/10.1016/j.jfca.2017.04.008

Danielie, P.P., Lussiana, C., Gasco, L., Amici, A. & Ronchi, B., 2019. The effect of diet formulation on the yield, proximate composition, and fatty acid profile of the black soldier fly (*Hermetia illucens* L.) prepupae intended for animal feed. Animals. 9, 178. doi:10.3390/ani9040178

El-Dakar, M.A., Ramzy, R.R. & Ji, H., 2021a. Influence of substrate inclusion of quail manure on the growth performance, body composition, fatty acid and amino acid profiles of black soldier fly larvae (*Hermetia illucens*). Sci. Total Environ. 772, 145528. https://doi.org/10.1016/j.scitotenv.2021.145528

El-Dakar, M.A., Ramzy, R.R., Ji, H. & Plath, M., 2020. Bioaccumulation of residual omega-3 fatty acids from industrial Schizochytrium microalgal waste using black soldier fly (*Hermetia illucens*) larvae. J. Clean. Prod. 268, 122288. https://doi.org/10.1016/j.jclepro.2020. 122288

El-Dakar, M.A., Ramzy, R.R., Wang, D. & Ji, H., 2021b. Sustainable management of Se-rich silkworm residuals by black soldier flies larvae to produce a high nutritional value and accumulate ω-3 PUFA. Waste Manag. 124, 72-81. https://doi.org/10.1016/j.wasman.2021.01.040

Ewald, N., Vidakovic, A., Langeland, M., Kiessling, A., Sampels, S. & Lalander, C., 2020. Fatty acid composition of black soldier fly larvae (*Hermetia illucens*) - possibilities and limitations for modification through diet. Waste Manag. 102, 40-47 https://doi.org/10.1016/j.wasman.2019.10.014

Fischer, H., Roomano, N. & Sinha, A.K., 2021. Conversion of spent coffee and donuts by black soldier fly (*Hermetia illucens*) larvae into potential resources for animal and plant farming. Insects. 12, 332. https://doi.org/10.3390/insects12040332

Galassi, G., Jucker, C., Parma, P., Lupi, D., Crovetto, G.M., Savoldelli, S. & Colombini, S., 2021. Impact of Agro-industrial byproducts on bioconversion, chemical composition, in vitro digestibility, and microbiota of the black soldier fly (Diptera: Stratiomyidae) larvae. J. Insect Sci. 21, 1-10. https://doi.org/10.1093/jisesa/ieaa148

Stellenbosch University https://scholar.sun.ac.za

Gao, Z., Wang, W., Lu, X., Zhu, F., Liu, W., Wang, X. & Lei, C., 2019. Bioconversion performance and life table of black soldier fly (*Hermetia illucens*) on fermented maize straw. J. Clean. Prod. 230, 974-980 https://doi.org/10.1016/j.jclepro.2019.05.074

Guil-Guerrero, J.L., Sánchez-Muros, M.J., Fabrikov, D., Rodríguez-Lozano, B., González-Fernández, M.J., Lyashenko, S. & Barroso, F.G., 2020. *Hermetia illucens* larvae as a living bioreactor for simultaneous food by-products recycling and useful oil production. J. Am. Oil Chem. Soc. 97, 717-727. https://doi.org/10.1002/aocs.12370

Hoc, B., Francis, F., Carpentier, J., Mostade, L., Blecker, C., Purcaro, G. & Caparros Megido, R., 2021a. ω3-enrichment of *Hermetia illucens* (L. 1758) prepupae from oilseed byproducts. J. Saudi Soc. Agric. Sci. 20, 155-163. https://doi.org/10.1016/j.jssas.2021.01.001

Hoc, B., Tomson, T., Malumba, P., Blecker, C., Jijakli, M.H., Purcaro, G., Francis, F. & Caparros Megido, R., 2021b. Production of rainbow trout (*Oncorhynchus mykiss*) using black soldier fly (*Hermetia illucens*) prepupae-based formulations with differentiated fatty acid profiles. Sci. Total Environ. 794, 148647. https://doi.org/10.1016/j.scitotenv.2021.148647

Jucker, C., Erba, D., Leonardi, M.G., Lupi, D. & Savoldelli, S., 2017. Assessment of vegetable and fruit substrates as potential rearing media for *Hermetia illucens* (Diptera: Stratiomyidae) larvae. Environ. Entomol. 46, 1415-1423. https://doi.org/10.1093/ee/nvx154

Kenar, J.A., Moser, B.R. & List, G.R., 2017. Chapter 2: Naturally occurring fatty acids: sources, chemistry, and uses. In: Fatty acids. 1st Edition. Ed: Ahmad, M.U., AOCS Press, London, United Kingdom. pp.24-71. https://doi.org/10.1016/B978-0-12-809521-8.00002-7

Liland, N.S., Biancarosa, I., Araujo, P, Biemans, D., Bruckner, C.G., Woogbø, R., Torstensen, B.E. & Lock, E.J., 2017. Modulation of nutrient composition of black soldier fly (*Hermetia illucens*) larvae by feeding seaweed-enriched media. PLoS One. 12. https://doi.org/10.1371/journal.pone.0183188

Meneguz, M., Schiavone, A., Gai, F., Dama, A., Lussiana, C., Renna, M. & Gasco, L., 2018. Effect of rearing substrate on growth performance, waste reduction efficiency and chemical composition of black soldier fly (*Hermetia illucens*) larvae. J. Sci. Food Agric. 98, 5776–5784. https://doi.org/10.1002/jsfa.9127

Oonincx, D.G.A.B., van Broekhoven, S., van Huis, A. & van Loon, J.J.A., 2015. Feed conversion, survival and development, and composition of four insect species on diets composed of food by-products. PLoS One. 10. https://doi.org/10.1371/journal.pone.0144601

Stellenbosch University https://scholar.sun.ac.za

Oonincx, D.G.A.B., Laurent, S., Veenenbos, M.E. & van Loon, J.J.A., 2020. Dietary enrichment of edible insects with omega 3 fatty acids. Iinsect Sci. 27, 500-509. https://doi.org/10.1111/1744-7917.12669

Romano, N., Fischer, H., Kumar, V., Francis, S.A. & Sinha, A.K., 2021. Productivity, conversion ability, and biochemical composition of black soldier fly (*Hermetia illucens*) larvae fed with sweet potato, spent coffee or dough. Int. J. Trop. Insect Sci. https://doi.org/10.1007/s42690-021-00532-5

Saadoun, J.H., Montevecchi, G., Zanasi, L., Bortolini, S., Macavei, L.I., Masino, F., Maistrello, L. & Antonelli, A., 2020. Lipid profile and growth of black soldier flies (*Hermetia illucens*, Stratiomyidae) reared on by-products from different food chains. J. Sci. Food Agric. 100, 3648-3657. https://doi.org/10.1002/jsfa.10397

Schreven, S.J.J., Yener, S., van Valenberg, H.J.F., Dicke, M. & van Loon, J.J.A., 2021. Life on a piece of cake: performance and fatty acid profiles of black soldier fly larvae fed oilseed by-products. J. Insects as Food Feed. 7, 35-49. https://doi.org/ 10.3920/JIFF2020.0004

Somroo, A.A., ur Rehman, K., Zheng, L., Cai, M., Xiao, X., Hu, S., Mathys, A., Gold, M., Yu, Z. & Zhang, J., 2019. Influence of Lactobacillus buchneri on soybean curd residue co-conversion by black soldier fly larvae *(Hermetia illucens*) for food and feedstock production. Waste Manag. 86, 114-122. https://doi.org/10.1016/j.wasman.2019.01.022

St-Hilaire, S., Cranfill, K., McGuire, M.A., Mosley, E.E., Tomberlin, J.K., Newton, L., Sealey, W., Sheppard, C. & Irving, S., 2007. Fish offal recycling by black soldier fly produces a foodstuff high in omega-3 fatty acids. J. World Aquac. Soc. 38, 197-202. https://doi.org/10.1111/j.1749-7345.2007.00101.x

Starcevic, K., Lozica, L., Gavrilovic, A., Heruc, Z. & Masek, T., 2019. Fatty acid plasticity of black soldier fly (*Hermetia Illucens*) larvae reared on alternative feeding media: crude olive cake and processed animal protein. J. Anim. Feed Sci. 28, 374-382. https://doi.org/10.22358/jafs/114434/2019

Truzzi, C., Giorgini, E., Annibaldi, A., Antonucci, M., Illuminati, S., Scarponi, G., Riolo, P., Isidoro, N., Conti, C., Zarantoniello, M., Cipriani, R. & Olivotto, I., 2020. Fatty acids profile of black soldier fly (*Hermetia illucens*): influence of feeding substrate based on coffee-waste silverskin enriched with microalgae. Anim. Feed Sci. Technol. 259, 114309. https://doi.org/10.1016/j.anifeedsci.2019.114309

Wang, S.Y., Wu, L., Li, B. & Zhang, D., 2020. Reproductive potential and nutritional composition of *Hermetia illucens* (Diptera: Stratiomyidae) prepupae reared on different organic wastes. J. Econ. Entomol. 113, 527-537. https://doi.org/10.1093/jee/toz296

Stellenbosch University https://scholar.sun.ac.za

CHAPTER 6

Meta-analyses of polyunsaturated fatty acids

Naturally occurring polyunsaturated fatty acids (PUFAs) contain two to six double bonds. In plants PUFAs generally have a maximum of three double bonds, but in algae and fish they can contain up to six. These fatty acids have a further classification into omega-3 (n-3) and omega-6 (n-6) fatty acids, which referred to the double bond carbon in the chain that is closest to the terminal methyl end. Humans and animals do not have the ability to biosynthesize these fatty acids and therefore they have to be accumulated through the diet as they are essential for proper functioning and health.

As with the meta-analyses performed for the saturated fatty acids, data from 29 trials that originated from 26 studies were used to perform meta-analyses (**Error! Reference source not found.** in Appendix A). Sixteen PUFAs were reportedly present in the BSFL fatty acid profile by either one or multiple studies (Table 6.6). Only one of the PUFAs, linoleic acid, was always present in the larvae. Meta-analyses were performed on data regarding nine of the identified PUFAs. The other PUFAs were excluded for the meta-analysis as they were not detected in the BSFL fatty acid profile by a sufficient number of trials. Meta-analyses were also performed on the total PUFA content as well as the total omega-6 and omega-3 fatty acid content, respectively.

Stellenbosch University https://scholar.sun.ac.za

Table 6.6: List of polyunsaturated fatty acids identified as present in the black soldier fly larval fatty acid profile

Common name	Preferred IUPAC	Omega nomenclature	Inclusion in meta-analysis
Linoleic acid	all-cis-9,12-octadecadienoic acid	C18:2n-6	Yes
Linolelaidic acid	Trans,trans-9,12-octadecadienoic acid	C18:2 trans	No
α-Linolenic acid	all-cis-9,12,15-octadecatrienoic acid	C18:3n-3	Yes
γ-Linolenic acid	all-cis-6,9,12-octadecatrienoic acid	C18:3n-6	Yes
Stearidonic acid	all-cis-6,9,12, 15-octadecatetraenoic acid	C18:4n-3	Yes
Eicosadienoic acid	all-cis-11,14-eicosadienoic acid	C20:2n-6	Yes
Eicosatrienoic acid	All-cis-11,14,17-eicosatrienoic acid	20:3n-3	No
Dihomo-γ-linolenic acid	All-cis-8,11,14-eicosatrienoic acid	C20:3n-6	No
Eicosatetraenoic acid	all-cis-8,11,14,17-eicosatetraenoic acid	C20:4n-3	No
Arachidonic acid	all-cis-5,8,11,14-eicosatetraenoic acid	C20:4n-6	Yes
Eicosapentaenoic acid	all-cis-5,8,11,14,17-eicosapentaenoic acid	C20:5n-3	Yes
Docosadienoic acid	all-cis-13,16-docosadienoic acid	C22:2n-6	No
Adrenic acid	all-cis-7,10,13,16-docosatetraenoic acid	C22:4n-6	No
Docosapentaenoic acid	all-cis-7,10,13,16,19-docosapentaenoic acid	C22:5n-3	No
Docosapentaenoic acid	all-cis-4,7,10,13,16-docosapentaenoic acid	C22:5n-6	Yes
Docohexaenoic acid	all-cis-4,7,10,13,16,19-docosahexaenoic acid	C22:6n-3	Yes

6.1 Summary statistics

The summary statistics regarding the BSFL fatty acid polyunsaturated fatty acids (PUFAs) consisted of the lowest observed concentrations, the highest observed concentration, and the largest difference in concentration of an individual fatty acid (Table 6.). Only linoleic acid was found to always be present in the BSFL fatty acid profile. Therefore, the lowest concentration for all the other PUFAs was noted as zero. Linoleic acid was also

Stellenbosch University https://scholar.sun.ac.za

demonstrated to have the highest observed concentration among the individual fatty acids with 37.40%, which is more than 10% higher than any of observed concentrations for the other PUFAs (Saadoun *et al.*, 2020). The total amount of PUFA in the BSFL fatty acid profile exhibited a range from 2.80% (Jucker *et al.*, 2017) to 42.10% (Jucker *et al.*, 2017).

Table 6.2: Summary statistic regarding levels of individual polyunsaturated fatty acids (PUFAs) in black soldier fly larval fatty acid profile (in % of total fatty acids)

Fatty acid	**Lower concentration limit (mean ± SD)**	**Higher concentration limit (mean ± SD)**	**Largest change difference in concentration (mean [CI])**
Linoleic acid (C18:2n-6)	0.70 ± 0.10 (Guil-Guerrero *et al.*, 2020)	37.40 ± 0.30 (Saadoun *et al.*, 2020)	26.90 [26.42, 27.38] (Saadoun *et al.*, 2020)
α-Linolenic acid (C18:3n-3)	0*	22.10 ± 1.07 (El-Dakar *et al.*, 2021b)	20.43 [19.37, 21.49] (El-Dakar *et al.*, 2021b)
γ-Linolenic acid (C18:3n-6)	0*	1.90 ± 0.60 (Truzzi *et al.*, 2020)	1.80 [1.49, 2.11] (Truzzi *et al.*, 2020)
Stearidonic acid (C18:4n-3)	0*	2.90 ± 0.40 (Guil-Guerrero *et al.*, 2020)	2.40 [1.89, 2.91] (Guil-Guerrero *et al.*, 2020)
Eicosadienoic acid (C20:2n-6)	0*	0.17 ± 0.05 (Schreven *et al.*, 2021)	0.10 [0.02, 0.18] (Schreven *et al.*, 2021)
Arachidonic acid (C20:4n-6)	0*	5.50 ± 0.20 (Guil-Guerrero *et al.*, 2020)	3.80 [3.41, 4.19] (Truzzi *et al.*, 2020)
Eicosapentaenoic acid (C20:5n-3)	0*	11.70 ± 0.10 (Truzzi *et al.*, 2020)	11.10 [11.03, 11.17] (Truzzi *et al.*, 2020)
Docosapentaenoic acid (C22:5n-6)	0*	0.84 ± 0.50 (Schreven *et al.*, 2021)	0.77 [0.27, 1.27] (Schreven *et al.*, 2021)

Stellenbosch University https://scholar.sun.ac.za

Table 6.2: Continued

Fatty acid	Lower concentration limit (mean ± SD)	Higher concentration limit (mean ± SD)	Largest change difference in concentration (mean [CI])
Docohexaenoic acid (C22:6n-3)	0*	16.70 ± 0.30 (Truzzi *et al.*, 2020)	16.20 [16.04, 16.36] (Truzzi *et al.*, 2020)
Total n-3 fatty acids	0*	16.50 ± 2.50 (Ewald *et al.*, 2020)	14.90 [s12.07, 17.73] (Ewald *et al.*, 2020)
Total n-6 fatty acids	0*	21.10 ± 1.40 (Liland *et al.*, 2017)	18.10 [17.41, 18.79] (Jucker *et al.*, 2017)
Total PUFA	2.80 ± 0.10 (Jucker *et al.*, 2017)	38.90 ± 0.40 (Saadoun *et al.*, 2020)	27.10 [26.38, 27.82] (Saadoun *et al.*, 2020)

* Fatty acid was absent in black soldier fly larvae in some included trials.
All values presented as % of total fatty acids.
SD – standard deviation.
CI – confidence interval.

The fatty acid profile data collected for the purpose of performing meta-analyses was in the form of the concentrations of the individual fatty acids in respect to the BSFL fatty acid profile (mean ± SD) and the fatty acid profile analysis sample sizes. Some of the studies reported the standard errors instead of the standard deviations. The standard deviations were then calculated using Equation 6.12.

Equation 6.12: Standard deviation calculation

$$Standard\ deviation = standard\ error \times \sqrt{sample\ size}$$

Preliminary meta-analyses were performed on the data, and it was found that the heterogeneity was so high for the meta-analyses to produce accurate results. The data was therefore transformed using the Freeman-Tukey double arcsine transformation (Freeman & Tukey, 1950). Equation 6.13 illustrates the transformation, where y_i is the original data point and $\hat{y}_i$ is the transformed data point.

Stellenbosch University https://scholar.sun.ac.za

Equation 6.13: Freeman-Tukey double arcsine transformation

$$\hat{y}_i = \arcsin\sqrt{y_i/(100+1)} + arcsin\sqrt{(y_i+1)/(100+1)}$$

The data transformation also changed the scale of the data, which was originally in % of total fatty acids. The meta-analysis results were reported on the transformed scale. It was decided to report in the transformed scale as a back-transformation of the results to the original scale had a high risk of producing misleading results.

The meta-analysis protocol was used to calculate the intervention effects, also known as the difference in means. These are visually represented in a forest plot. The intervention effects were weighted, and the average effect calculated. The statistical component of the meta-analysis consisted of tests for heterogeneity and a Z-test for the overall effect. The Chi^2 test was performed to evaluate if there as any heterogeneity of importance. The null hypothesis was that there was no observed heterogeneity of importance, and the alternative hypothesis was that there was heterogeneity that could have an impact on the results. The accompanying Tau^2 statistic represented the variance among the observed intervention effects and is incorporated into the weighting of the intervention effects by the meta-analysis software. The I^2 statistic represented the percentage of heterogeneity found among the intervention effects. This provided a scale that could be used to determine a cut-off point as to when the heterogeneity was too high for a meta-analysis to be justified. For the Z-test of the overall effect the null hypothesis was that there was no effect on the concentration of the individual PUFA due to the rearing substrate composition and that any difference in means was purely due to chance. The alternative hypothesis was that the intervention effect was indeed due to the effect of the rearing substrate composition.

6.2 Linoleic acid

Linoleic acid is an omega-6 fatty acid consisting of an 18 carbon-chain with two double bonds (C18:2 n-6). It is found in high concentrations in plant oils such as cottonseed, soybean and sunflower oil (Kenar *et al.*, 2017). It was the only PUFA that was found to be present in the BSFL fatty acid profile by all 29 trials in this study. The lowest observed concentration was 0.70% when the larvae were reared on coconut by-product, which had very low concentration of unsaturated fatty acids and very high concentrations of saturated fatty acids (Guil-Guerrero *et al.*, 2020). The highest observed concentration was 37.40% when the larvae were reared on tomato peels and seeds (Saadoun *et al.*, 2020). The same

Stellenbosch University https://scholar.sun.ac.za

study reported the largest difference in linoleic acid concentration (26.90%). This was also the largest difference found among any of the PUFAs.

The meta-analysis of linoleic acid with accompanying statistics and forest plot can be seen in Figure 6.. The Chi^2 test indicated that there was heterogeneity among the intervention effects (P = 0.007). The Tau^2 statistic, which is an indication of the variation among the intervention effects, also indicated some heterogeneity. The I^2 statistic, which is an indication of the extent of the heterogeneity, indicated a moderate level of heterogeneity. It was however still acceptably low for the meta-analysis. This slight deviation from complete homogeneity can also be seen in the forest plot where there is a shift to the right and some intervention effect confidence intervals do not overlap. The Z-test for the overall effect produced a very low P-value (P < 0.00001). This indicated that the rearing substrate composition had an effect on the linoleic acid concentration and that the differences in concentrations were due to the investigated effect and not purely due to chance.

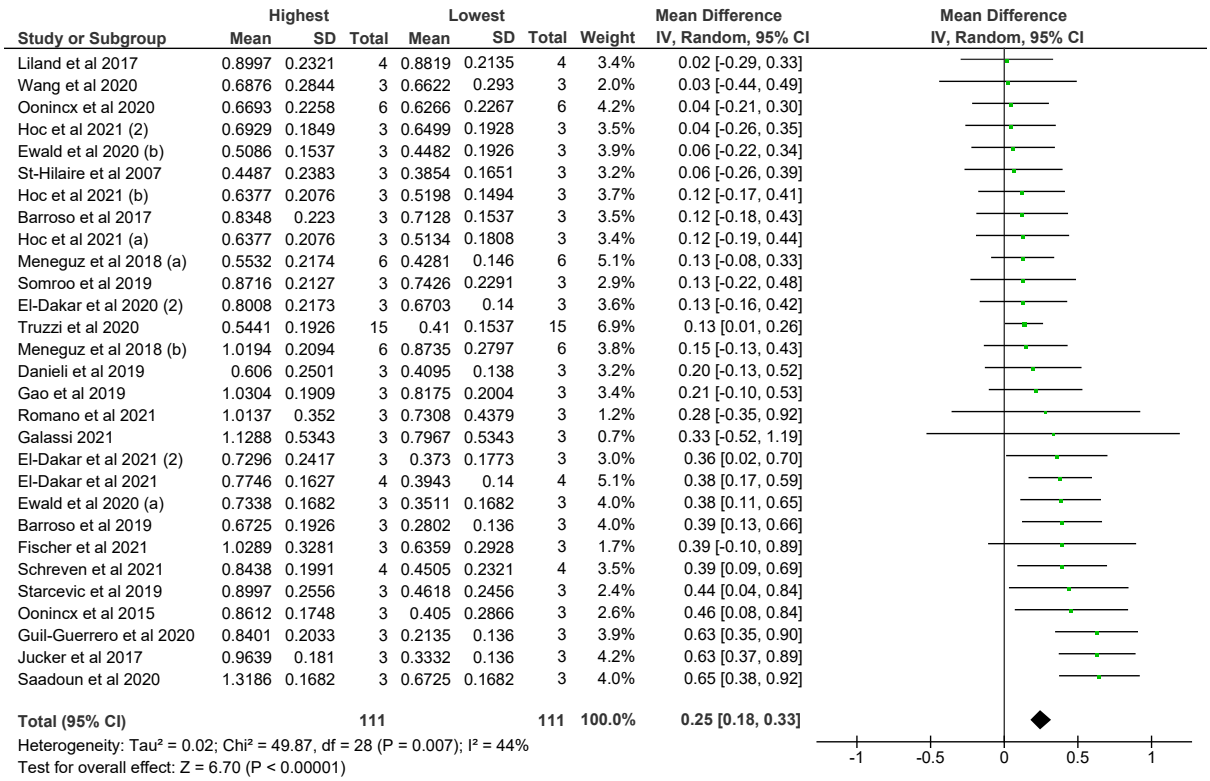


Figure 6.1: Software output of meta-analysis of linoleic acid concentration of black soldier fly larval fatty acid profile

Stellenbosch University https://scholar.sun.ac.za

6.3 α-Linolenic acid

α-Linolenic acid is an omega-3 PUFA consisting of an 18 carbon-chain with three double bonds (C18:3 n-3). It is found in most plant oils, such as soybean, canola, flax seed and soy, and some animal fats (Kenar *et al.*, 2017). It is considered an essential fatty acid for humans and animals as they do not have the ability to biosynthesize α-linolenic acid. It can then be converted into other longer chain PUFAs by liver desaturase and elongation enzymes. Linolenic acid has two isomers of which α-linolenic acid is naturally found in higher concentrations. Most of the trials reported it as present in the BSFL fatty acid profile, but as two reported it as absent the lowest concentration was noted as zero. The highest observed concentration was 22.10% when the larvae were reared on ground silkworm pupae, which is a by-product of the silk industry and is itself high in α-linolenic acid (El-Dakar *et al.*, 2021b). The same study found the largest difference in concentration, which was 20.43%. After linoleic acid, α-Linolenic acid was the PUFA that was observed to be found in the highest concentrations in BSFL.

The meta-analysis of the α-linolenic acid data is shown in Figure 6.. The statistical output regarding heterogeneity and the overall effect as well as the forest plot can also be seen in the figure. The Chi^2 test indicated that there was heterogeneity among the intervention effects that could influence the accuracy of the meta-analysis results as it had a very low P-value ($P < 0.00001$). The Tau^2 statistic indicated a level of variation among the effects. The I^2 statistic indicated that there was 64% heterogeneity, which is seen as substantial heterogeneity. The Z-test for the overall effect indicated that the rearing substrate composition had an effect on the concentration of α-linolenic acid in the BSFL as the P-value was very low ($P < 0.00001$). As previously mentioned, there was substantial heterogeneity among the intervention effects. Therefore, the meta-analysis results in terms of the calculated average intervention effect should be interpreted with the lowered accuracy in mind.

Stellenbosch University https://scholar.sun.ac.za

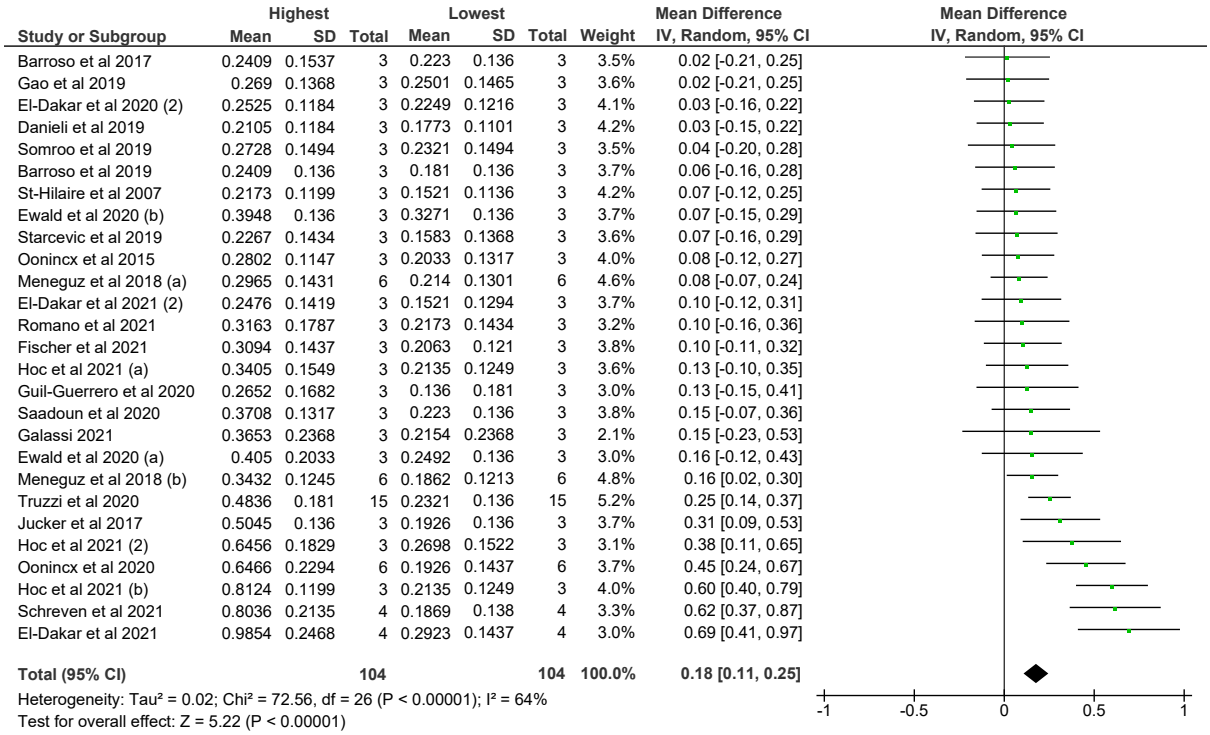


Figure 6.2: Software output of meta-analysis of α-linolenic acid concentration of black soldier fly larval fatty acid profile

6.4 γ-Linolenic acid

γ-Linolenic acid is the omega-6 isomer of linolenic acid (C18:3n-6). It is found in smaller quantities in nature compared to α-linolenic acid. In humans and animals, linoleic acid is converted into γ-linolenic acid as an intermediate step in the synthesis of arachidonic acid. Studies suggest that γ-linolenic acid could play a role in the prevention and alleviation of some diseases in humans. Only two of the trials reported γ-Linolenic acid as present in the BSFL fatty acid profile. This is in line with the assumption that it is rarely found in BSFL as opposed to α-linolenic acid, which was almost always detected in the larvae. The highest observed concentration was 1.90% when the larvae were reared on coffee silverskin, a by-product of coffee production, enriched with algae (Truzzi *et al.*, 2020). The same study reported the largest difference in concentration with a difference of 1.80%.

Figure 6. shows the meta-analysis performed on the γ-linolenic acid data along with its forest plot. The Chi^2 test indicated that there was no heterogeneity of importance among the intervention effects (P = 0.42). Both the Tau^2 statistic and the I^2 statistic also indicated that the intervention effects were sufficiently homogeneous so that it would not have an effect on the meta-analysis results. The Z-test for the overall effect indicated that the rearing substrate composition had an effect on the concentration of γ-Linolenic in the BSFL (P = 0.007).

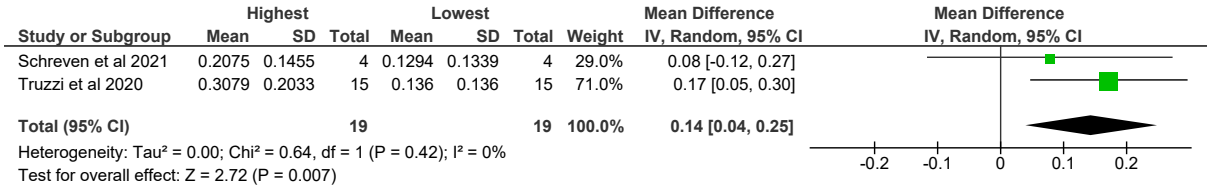


Figure 6.3: Software output of meta-analysis of γ-linolenic acid concentration of black soldier fly larval fatty acid profile

6.5 Stearidonic acid

Stearidonic acid is an omega-3 PUFA that is made up of a chain with 18 carbons and four double bonds (C18:4n-3). It is one of the fatty acids that are synthesized from linolenic acid by means of desaturase. Stearidonic acid was reported as present in the BSFL fatty acid profile by four of the trials. This indicates that it is not that common in the larvae. The highest observed concentration was 2.90% when the larvae were reared on a laying hen feed supplemented with 10% guar (Guil-Guerrero *et al.*, 2020). It should however be noted that none of the substrates used in this specific trial contained any detectable stearidonic acid. Therefore, the larvae biosynthesized the stearidonic acid from the linolenic acid available in the substrate. The largest difference in concentration reported by one trial was 2.40% (Guil-Guerrero *et al.*, 2020).

The meta-analysis result and the forest plot representation is seen in Figure 6.. The Chi^2 test indicated that there was no heterogeneity among the intervention effects (P = 0.87). This was also found for the Tau^2 statistic and the I^2 statistic. This suggested that there was no heterogeneity among the intervention effect that could influence the meta-analysis. The Z-test for the overall effect had a P-value slightly higher than the cut off value for significance (P = 0.08). This suggested that the observed intervention effect was likely due to chance rather than in rearing substrate composition. It should however be noted that the P-value was close to the significance cut off value, which was P = 0.05. This could indicate that there might be an observed effect. Data from more trials would likely give a better indication of the significance of an observed effect.

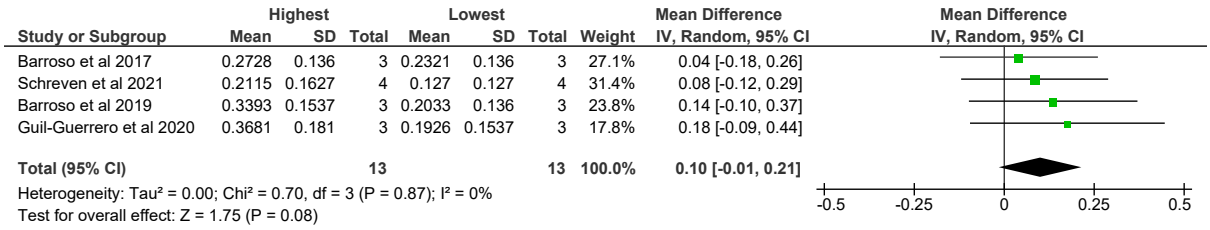


Figure 6.4: Software output of meta-analysis of stearidonic acid concentration of black soldier fly larval fatty acid profile

6.6 Eicosadienoic acid

Eicosadienoic acid is an omega-6 PUFA that consists of a 20 carbon-chain with two double bonds (C20:2n-6). It is occasionally found in small quantities (< 0.1% of total fatty acids) in plant oils and animal fats. The highest observed concentration was 0.17% when the larvae were reared on camelina pressed cake, which reportedly contained 1.39% eicosadienoic acid (Schreven *et al.*, 2021). The same study reported the largest difference in concentration, which was 0.10%.

The meta-analysis was performed on the eicosadienoic acid concentration data and is shown in Figure 6.. The heterogeneity was evaluated with the Chi^2 test, and the results suggested there was no heterogeneity (P = 0.99). The same conclusion could be made based on the Tau^2 statistic and the I^2 statistic, which both suggested that the intervention effects were homogeneous. The Z-test indicated that the effect was not significant and that the observed differences were likely due to chance rather than rearing substrate composition.

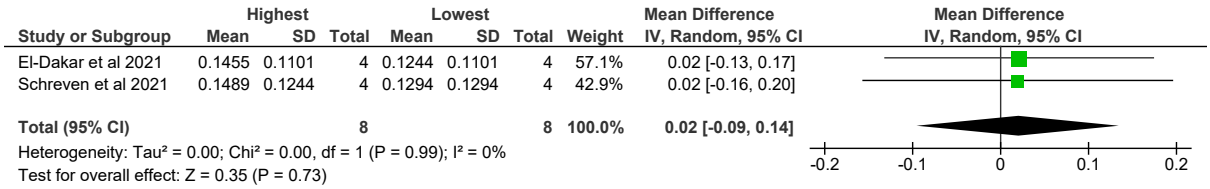


Figure 6.5: Software output of meta-analysis of eicosadienoic acid concentration of black soldier fly larval fatty acid profile

Stellenbosch University https://scholar.sun.ac.za

6.7 Arachidonic acid

Arachidonic acid is an omega-6 PUFA that consists of a 20 carbon-chain with four double bonds (C20:4n-6). It is occurs naturally in plant oils such as corn, soybean and sunflower oil (Kenar *et al.*, 2017). Although humans and animals have the ability to biosynthesize arachidonic acid from linoleic acid to a small extent, their predominant arachidonic acid source is meat and fish. Seven of the trials reported the presence of arachidonic acid in the BSFL fatty acid profile. The largest observed concentration was 5.50% when the larvae were reared on pork viscera (Guil-Guerrero *et al.*, 2020). The largest reported difference in concentration was 3.80% (Truzzi *et al.*, 2020).

Figure 6. illustrates the results of the meta-analysis performed on the arachidonic acid concentration data. The results are accompanied by a forest plot that illustrates the intervention effect confidence intervals. The Chi^2 test indicated that there was no heterogeneity as the P-value was larger than 0.05 (P = 0.18). The Tau^2 statistic also indicated homogeneity among the intervention effects. The I^2 statistic did indicate a small amount of heterogeneity (32%). This was however deemed not to be important. The forest plot suggests that the observed heterogeneity originated from the results of one trial as its confidence interval has a lack of overlap with the other confidence intervals. The Z-test for the overall effect suggested that the rearing substrate composition did indeed have a significant effect on the arachidonic acid concentration in the larvae (P = 0.03).

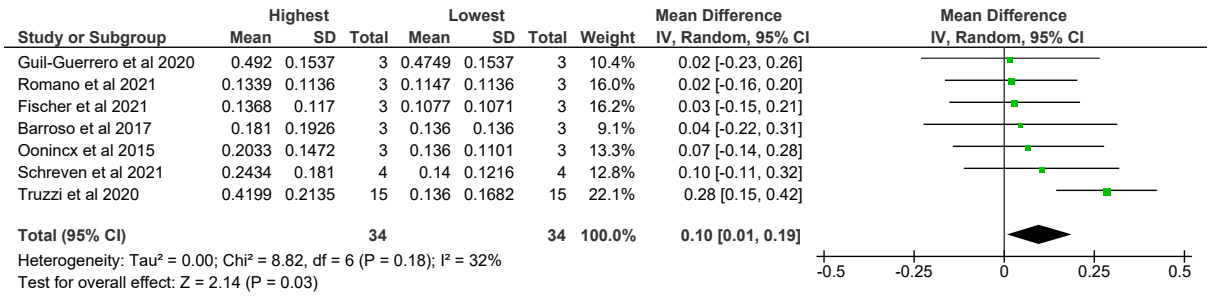


Figure 6.6: Software output of meta-analysis of arachidonic acid concentration of black soldier fly larval fatty acid profile

6.8 Eicosapentaenoic acid

Eicosapentaenoic acid (EPA) is an omega-3 PUFA made up of a 20 carbon-chain with five double bonds (C20:5n-3). It is considered an important fatty acid for proper human biological functioning and health. Humans main source of EPA is fish oil. Fish cannot however

biosynthesize EPA and rather accumulate it through the ingestion of algae, which have the ability to biosynthesize the fatty acid. Seven of the trials reported its presence in the BSFL fatty acid profile. The highest observed EPA concentration was 11.70% when the larvae were reared on coffee silverskin substituted with 25% algae, which as previously mentioned have the ability to biosynthesize EPA (Truzzi *et al.*, 2020). The same study reported the largest difference in EPA concentration, which was reported as 11.1%.

Error! Reference source not found. illustrates the second meta-analysis performed on the EPA concentration data. The results of the first meta-analysis can be found in Appendix B (Figure B 3). The Chi^2 test performed on the first meta-analysis data suggested that there was definitely heterogeneity among the observed intervention effects. The I^2 statistic also indicated that the heterogeneity was considerable (80%). The Tau^2 statistic suggested the same. Such a large amount of heterogeneity would most likely influence the results of the meta-analysis. The source of the heterogeneity was therefore investigated. It was found that the data from one trial was responsible for the heterogeneity (Truzzi *et al.*, 2020). A second meta-analysis was therefore performed on the EPA data while excluding the data from this particular trial. The trial data was excluded on the basis that it was the only trial that reared the larvae on a substrate with such high concentrations of EPA due to the inclusion of algae and that the difference between it and the other trials negatively affected the meta-analysis. The Chi^2 test resulting from the second meta-analysis suggested that the intervention effects were homogeneous (P = 0.83). The Z-test for the overall effect suggested that the rearing substrate composition had an effect on the EPA concentration of the larvae (P = 0.01). By comparing the average intervention effects calculated by the two meta-analyses performed on the EPA concentration data, it is visible that the excluded study had an effect on it.

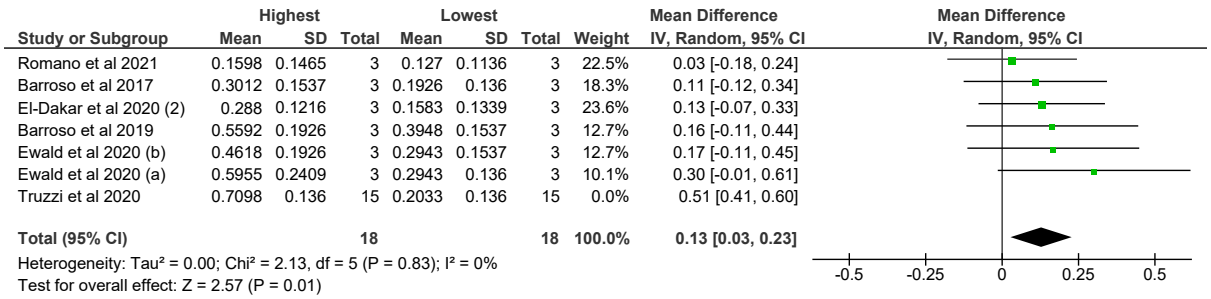


Figure 6.7: Software output of meta-analysis of eicosapentaenoic acid concentration of black soldier fly larval fatty acid profile

Stellenbosch University https://scholar.sun.ac.za

6.9 Docosapentaenoic acid

Docosapentaenoic acid (DPA) is an omega-6 PUFA that consists of a 22 carbon-chain with five double bonds (C22:5n-6). Only two of the trials reported the presence of DPA in the BSFL fatty acid profile. The highest observed concentration was 0.84% when the larvae were reared on chicken feed substituted with 25% *crambe* pressed cake (Schreven *et al.*, 2021). The feed itself did not contain any DPA, but it did however contain precursor fatty acids for its biosynthesis. The largest difference in concentration, which was 0.77%, was reported by the same study.

The meta-analysis performed on the DPA concentration data is illustrated in Figure 6.. The Chi^2 test suggested that there was no heterogeneity among the intervention effects (P = 0.92). The homogeneity was confirmed by the Tau^2 statistic and the I^2 statistic. The Z-test for the overall effect indicated that the difference in concentrations were most likely due to chance rather than the rearing substrate composition (P = 0.34).

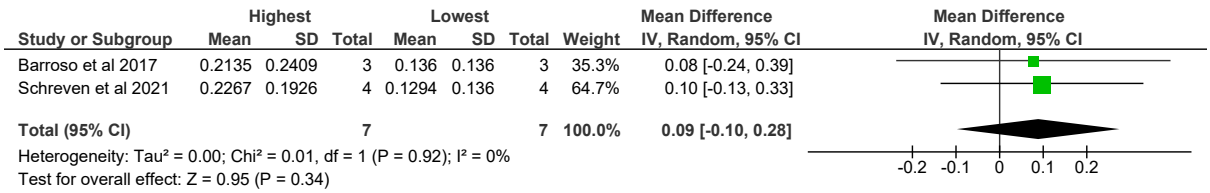


Figure 6.8: Software output of meta-analysis of docosapentaenoic acid concentration of black soldier fly larval fatty acid profile

6.10 Docohexaenoic acid

Docohexaenoic acid (DHA) is an omega-3 PUFA that consists of a 22 carbon-chain with five double bonds (C22:6n-3). Research has suggested an association between DHA and human health due to its possible role in the prevention of various diseases. The main source of DHA for humans and animals is fish oil. Six trials reported it to be present in the BSFL fatty acid profile. The highest observed concentration was 16.70% when the larvae were reared on coffee silverskin substituted with 20% algae, which biosynthesize DHA and can contain high concentrations of the fatty acid (up to 80% of total FA) (Truzzi *et al.*, 2020). The same study reported the largest difference in concentration with 16.20%.

Appendix B shows the results of the preliminary meta-analysis performed on the DHA concentration data (Figure B 4). Its Chi^2 test indicated that there was significant heterogeneity among the intervention effects (P < 0.00001). The I^2 statistic indicated that there was 89% heterogeneity, which is seen as considerable. Due to the high percentage of heterogeneity, the source was investigated. The forest plot suggested that one specific trial was the source of the heterogeneity (Truzzi *et al.*, 2020). This trial was also the source of heterogeneity for the meta-analysis of the EPA concentration. The rearing substrate used in this trial was much higher in DHA and EPA than any of the other trials. A second meta-analysis was performed on the data that excluded this trial data with the aim of calculating more accurate results. The second meta-analysis is shown in Figure 6.. The Chi^2 test performed for the second meta-analysis showed that excluding the one trial, reduced the heterogeneity

(P = 0.87). The I^2 statistic and the Tau^2 statistic indicated no heterogeneity in the second meta-analysis. The Z-test for the overall effect indicated that the rearing substrate composition had a significant effect on the DHA concentration of the larvae.

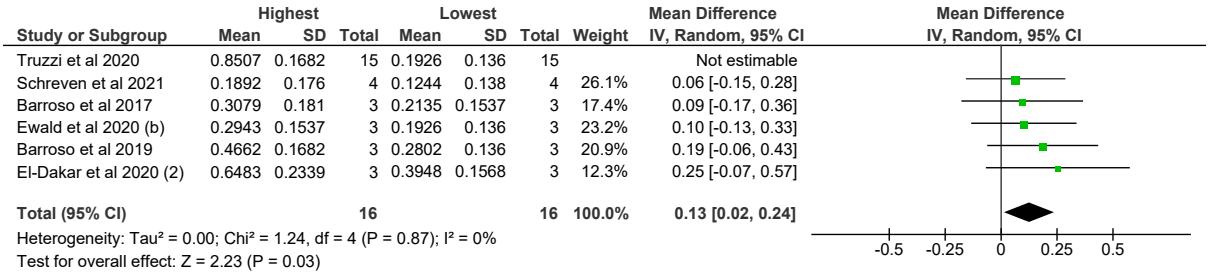


Figure 6.9: Software output of meta-analysis of docohexaenoic acid concentration of black soldier fly larval fatty acid profile

6.11 Total omega-6 and omega-3 fatty acid concentration

Polyunsaturated fatty acids of importance can generally be subdivided into two categories: the omega-6 (n-6) and omega-3 (n-3) fatty acids. The designations of n-6 and n-3 refer to the position of the double bond carbon that is closest to the terminal methyl end rather than the systematic numbering which starts at the carboxyl end (Kenar *et al.*, 2017). There are two PUFAs that are considered essential fatty acids for humans and animals as they lack the ability to biosynthesise these fatty acids successfully. These are linoleic acid (C18:2 n-6) and α-linolenic acid (C18:3 n-3). Linoleic acid and α-linolenic acid are then converted into other PUFAs in the body through desaturase and elongation enzymes reactions to meet the biological requirements for the other PUFAs. This process however has low efficiency. Therefore, the main sources of the various omega-6 and omega-3 fatty acids are still the

Stellenbosch University https://scholar.sun.ac.za

diet. Omega-6 and omega-3 fatty acids utilize the same enzymes during the biosynthesis process and are consequently competing substrates. Therefore, the total amount of omega-6 and omega-3 fatty acids, and especially the ratio of the two, play an important role in evaluating the nutritional value of food or feed. Recently the recommendation has been to aim for a ratio close to 1:1 of omega-6 to omega-3 fatty acids. Western diets are generally deficient in omega-3 and have excessive amounts of omega 6, which leads to a ratio as high as 15:1 to 16.7:1. Ratios as high as this have been associated with an increased risk of various diseases, including cardiac and pulmonary diseases.

Understanding the influence of the rearing substrate composition on the total amount of omega-6 and omega-3 fatty acids in the BSFL could assist in the formulation of rearing substrates that can improve the ratio and add to the nutritional value of the larvae. Meta-analyses were performed on the data regarding the total amount of omega-6 and omega-3 fatty acids in the BSFL fatty acid profile.

6.11.1 Omega-6 fatty acids

The highest observed concentration of omega-6 fatty acids was 21.10% when the larvae were reared on ground brown algae (Liland *et al.*, 2017). The largest reported difference in its concentration was 18.10% (Jucker *et al.*, 2017).

The results of the meta-analysis performed on the omega-6 fatty acid concentration data is shown in Figure 6.. The Chi^2 test indicated that there was heterogeneity among the intervention effects that should be addressed (P = 0.03). The Tau^2 statistic indication some variation and the I^2 statistic indicated that there was 59% heterogeneity among the intervention effects, which is considered a substantial amount of heterogeneity. The Z-test for the overall effect suggested that the rearing substrate composition had an effect on the concentration of omega-6 fatty acids in the BSFL fatty acid profile (P = 0.006). The suggested significant effect should however be interpreted conservatively as the heterogeneity tests indicated variation that could have influenced the effect size.

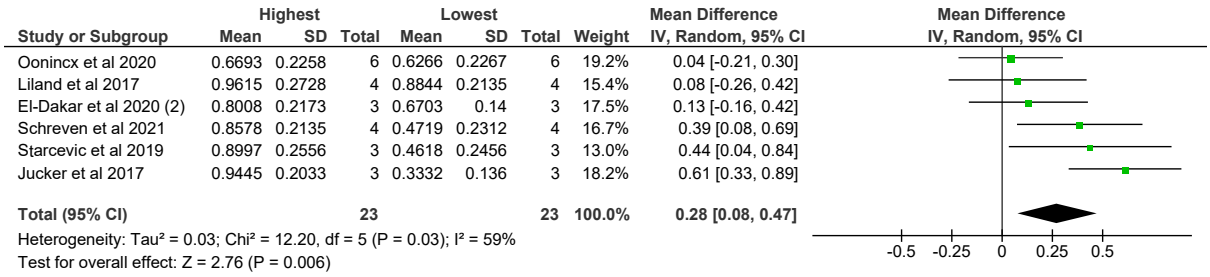


Figure 6.10: Software output of meta-analysis of total omega 6 fatty acids concentration of black soldier fly larval fatty acid profile

6.11.2 Omega-3 fatty acids

The highest observed concentration of omega-3 fatty acids was 16.50% when the larvae were reared on ensiled mussels, which was reportedly high in omega-3 fatty acids (44.90%) (Ewald *et al.*, 2020). The largest difference in omega-3 fatty acid concentration was 14.90% and was reported by the same study. The observed difference in omega-3 fatty acid concentration in the larvae mirrored an observed difference in omega-3 fatty acid concentration in the substrates.

Figure 6. illustrates the meta-analysis performed on the omega-3 fatty acid concentration data. The Chi^2 test indicated that there was no heterogeneity that needed to be addressed (P = 0.06). The P-value was however close to the cut off value and therefore the other statistics were also evaluated. The Tau^2 statistic indicated some heterogeneity and the I^2 statistic indicated 51% heterogeneity, which is seen as moderate. The Z-test for the overall effect suggested that the rearing substrate composition had an effect on the omega-3 fatty acid concentration of the larvae as the P-value was very low (P < 0.00001).

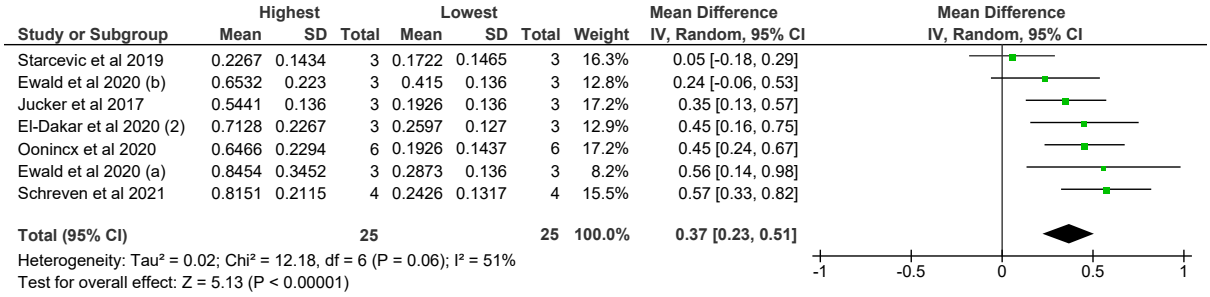


Figure 6.11: Software output of meta-analysis of total omega 3 fatty acids concentration of black soldier fly larval fatty acid profile

Stellenbosch University https://scholar.sun.ac.za

6.12 Total polyunsaturated fatty acids

The broadest way to categorise fatty acids are by grouping them based on saturation. Thereby obtaining two groups: saturated fatty acids (SFAs) and unsaturated fatty acids (UFAs). This categorisation indicates the level of saturation of a fatty acid profile. Unsaturated fatty acids are then subdivided into monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). This is done because of the major functional and structural differences between MUFAs and PUFAs. Reporting the total amount of PUFA therefore does not only describe the level of saturation of a fatty acid profile, but also indicates to structural and functional characteristics of the fatty acid profile. The total amount of PUFA was however not reported by all the trials in this study. Data regarding this concentration was collected from 20 of the included trials. The lowest observed PUFA concentration was 2.80% when the larvae were reared on fruit (Jucker *et al.*, 2017). The specific treatment group was also characterised by a high concentration of saturated fatty acids (86.00%). The highest observed PUFA concentration was 38.90%, mostly made up of linoleic acid, when the larvae were reared on tomato peels and seeds (Saadoun *et al.*, 2020). The largest difference in PUFA concentration was 27.10% (Saadoun *et al.*, 2020).

The results of the PUFA concentration meta-analysis are illustrated in Figure 6.. The Chi^2 test indicated that there was no heterogeneity among the intervention effects that needed to be addressed ($P = 0.15$). The Tau^2 statistic indicated a small amount of heterogeneity and the I^2 statistic indicated 25% heterogeneity. This amount of heterogeneity is however still seen as not important. The Z-test for the overall effect indicated that the rearing substrate composition had an effect on the PUFA concentration of the BSFL fatty acid profile as the P-value was very low ($P < 0.00001$).

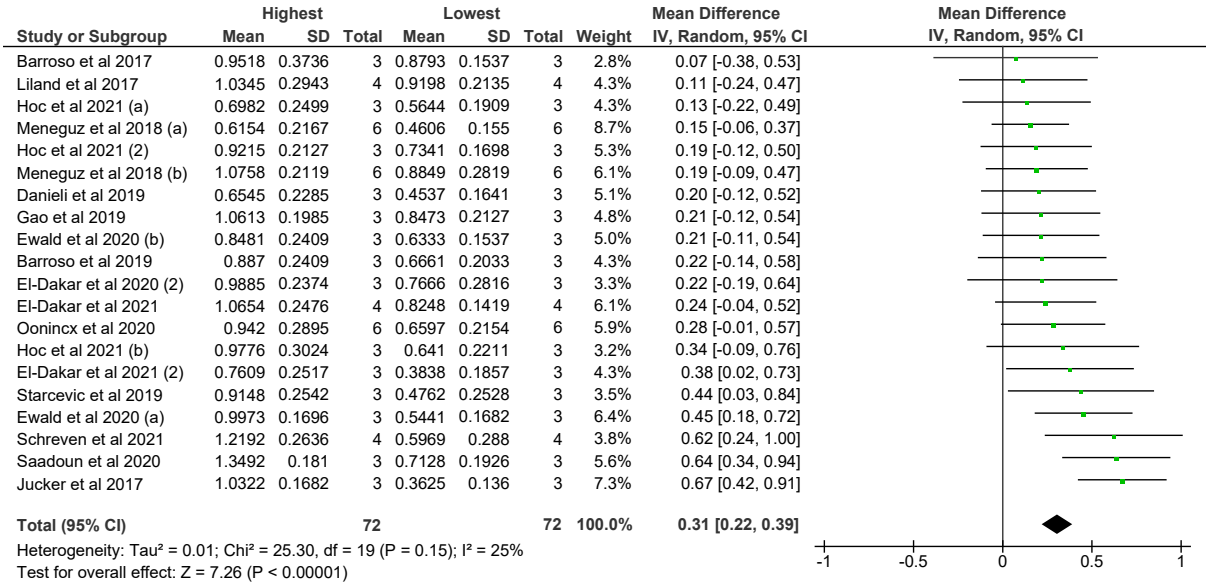


Figure 6.12: Software output of meta-analysis of the total polyunsaturated fatty acid concentration of black soldier fly larval fatty acid profile

6.13 Discussion

The meta-analyses performed on the concentration of the individual polyunsaturated fatty acids (PUFAs) and the categories calculated the significance of the observed effect as well as the heterogeneity among the observed intervention effects. The meta-analyses also calculated the average effect size as means and accompanying confidence intervals, which can be seen in Table 6.7. These average effect sizes were expressed on the scale of the transformed data. Back-transformation to the original scale of concentration was considered, but literature suggested that this could lead to misleading results. Therefore, the results were interpreted on the transformed scale.

Based on the average effect sizes the rearing substrate composition had the largest effect on the linoleic acid concentration in the BSFL fatty acid profile. Linoleic acid is the shortest PUFA that was identified as present in the BSFL fatty acid profile and can be used as a substrate for the biosynthesis of longer PUFAs. The rearing substrate composition also had a relatively large effect of both isomers of linolenic acid (α and γ). Three of the other PUFAs were found to be significantly affected by the rearing substrate composition, however not to the extent as was found to linoleic and linolenic acid. Arachidonic acid, which was significantly affected, is also known to be used as a substrate for the biosynthesis of other PUFAs. The other two PUFAs that were significantly affected were EPA and DHA. Black soldier fly larvae do not have the ability to biosynthesize these fatty acids and therefore it

Stellenbosch University https://scholar.sun.ac.za

was coherent that they were significantly affected by the rearing substrate composition. The lack of significant effect found for the other three PUFAs could be an indication that the larvae biosynthesize the required amount of these fatty acids irrespective of the rearing substrate composition.

Table 6.7: Average effect size (with confidence intervals) of rearing substrate composition on the different individual polyunsaturated fatty acid concentrations calculated by the meta-analyses

Fatty acid	Average effect size (with CI)	Z-test P-value	Chi^2 P-value	Tau^2	I^2
Linoleic acid	0.25 [0.18, 0.33]	<0.00001	0.007	0.02	44%
α-Linolenic acid	0.18 [0.11, 0.25]	<0.00001	<0.00001	0.02	64%
γ-Linolenic acid	0.14 [0.04, 0.25]	0.007	0.42	0.00	0%
Stearidonic acid	0.10 [-0.01, 0.21]	0.08	0.87	0.00	0%
Eicosadienoic acid	0.02 [-0.09, 0.14]	0.73	0.99	0.00	0%
Arachidonic acid	0.10 [0.01, 0.19]	0.03	0.18	0.00	32%
Eicosapentaenoic acid	0.13 [0.03, 0.23]	0.01	0.83	0.00	0%
Docosapentaenoic acid	0.09 [-0.10, 0.28]	0.34	0.92	0.00	0%
Docohexaenoic acid	0.13 [0.02, 0.24]	0.03	0.87	0.00	0%
Total n-6	0.28 [0.08, 0.47]	0.006	0.03	0.03	59%
Total n-3	0.37 [0.23, 0.51]	<0.00001	0.06	0.02	51%
Total PUFA	0.33 [0.21, 0.44]	<0.00001	0.15	0.01	25%

The total concentrations of omega-6 and omega-3 fatty acids in the BSFL fatty acid profile were both significantly affected. This is reasonable as numerous of the individual fatty acids that form part of these two categories were significantly affected. A larger average effect size was identified for omega-3 than omega-6. This also indicates that it could be possible to change the ratio of omega-6 and omega-3 fatty acids in the larvae to a desirable ratio for the larvae's intended purpose, be that animal feed or human food. The total PUFA concentration of the BSFL fatty acid profile was also found to be significantly affected by the rearing substrate composition. The table containing the average effect sizes represents guidelines that could be used to determine to what extent the concentrations of different PUFAs can be changed in the BSFL fatty acid profile though the formulation of rearing substrates with specific compositions.

Stellenbosch University https://scholar.sun.ac.za

6.14 References

Barroso, F.G., Sanchez-Muros, M.J., Rincon, M.A., Rodriguez-Rodriguez, M., Fabrikov, D., Morote, E. & Guil-Guerrero, J.L., 2019. Production of n-3-rich insects by bioaccumulation of fishery waste. J. Food Compos. Anal. 82, 103237. https://doi.org/10.1016/j.jfca.2019.103237

Barroso, F.G., Sánchez-Muros, M., Segura, M., Morote, E., Torres, A., Ramos, R. & Guil, J.L., 2017. Insects as food: enrichment of larvae of *Hermetia illucens* with omega 3 fatty acids by means of dietary modifications. J. Food Compos. Anal. 62, 8-13. https://doi.org/10.1016/j.jfca.2017.04.008

Danielie, P.P., Lussiana, C., Gasco, L., Amici, A. & Ronchi, B., 2019. The effect of diet formulation on the yield, proximate composition, and fatty acid profile of the black soldier fly (*Hermetia illucens* L.) prepupae intended for animal feed. Animals. 9, 178. doi:10.3390/ani9040178

El-Dakar, M.A., Ramzy, R.R. & Ji, H., 2021a. Influence of substrate inclusion of quail manure on the growth performance, body composition, fatty acid and amino acid profiles of black soldier fly larvae (*Hermetia illucens*). Sci. Total Environ. 772, 145528. https://doi.org/10.1016/j.scitotenv.2021.145528

El-Dakar, M.A., Ramzy, R.R., Ji, H. & Plath, M., 2020. Bioaccumulation of residual omega-3 fatty acids from industrial Schizochytrium microalgal waste using black soldier fly (*Hermetia illucens*) larvae. J. Clean. Prod. 268, 122288. https://doi.org/10.1016/j.jclepro.2020. 122288

El-Dakar, M.A., Ramzy, R.R., Wang, D. & Ji, H., 2021b. Sustainable management of Se-rich silkworm residuals by black soldier flies larvae to produce a high nutritional value and accumulate ω-3 PUFA. Waste Manag. 124, 72-81. https://doi.org/10.1016/j.wasman.2021.01.040

Ewald, N., Vidakovic, A., Langeland, M., Kiessling, A., Sampels, S. & Lalander, C., 2020. Fatty acid composition of black soldier fly larvae (*Hermetia illucens*) - possibilities and limitations for modification through diet. Waste Manag. 102, 40-47 https://doi.org/10.1016/j.wasman.2019.10.014

Fischer, H., Roomano, N. & Sinha, A.K., 2021. Conversion of spent coffee and donuts by black soldier fly (*Hermetia illucens*) larvae into potential resources for animal and plant farming. Insects. 12, 332. https://doi.org/10.3390/insects12040332

Freeman, M.F. & Tukey, J.W. 1950. Transformations related to the angular and the square root. Ann. Mathematical Stat., 21, 607-611.

Stellenbosch University https://scholar.sun.ac.za

Galassi, G., Jucker, C., Parma, P., Lupi, D., Crovetto, G.M., Savoldelli, S. & Colombini, S., 2021. Impact of Agro-industrial byproducts on bioconversion, chemical composition, in vitro digestibility, and microbiota of the black soldier fly (Diptera: Stratiomyidae) larvae. J. Insect Sci. 21, 1-10. https://doi.org/10.1093/jisesa/ieaa148

Gao, Z., Wang, W., Lu, X., Zhu, F., Liu, W., Wang, X. & Lei, C., 2019. Bioconversion performance and life table of black soldier fly (*Hermetia illucens*) on fermented maize straw. J. Clean. Prod. 230, 974-980 https://doi.org/10.1016/j.jclepro.2019.05.074

Guil-Guerrero, J.L., Sánchez-Muros, M.J., Fabrikov, D., Rodríguez-Lozano, B., González-Fernández, M.J., Lyashenko, S. & Barroso, F.G., 2020. *Hermetia illucens* larvae as a living bioreactor for simultaneous food by-products recycling and useful oil production. J. Am. Oil Chem. Soc. 97, 717-727. https://doi.org/10.1002/aocs.12370

Hoc, B., Francis, F., Carpentier, J., Mostade, L., Blecker, C., Purcaro, G. & Caparros Megido, R., 2021a. ω3-enrichment of *Hermetia illucens* (L. 1758) prepupae from oilseed byproducts. J. Saudi Soc. Agric. Sci. 20, 155-163. https://doi.org/10.1016/j.jssas.2021.01.001

Hoc, B., Tomson, T., Malumba, P., Blecker, C., Jijakli, M.H., Purcaro, G., Francis, F. & Caparros Megido, R., 2021b. Production of rainbow trout (*Oncorhynchus mykiss*) using black soldier fly (*Hermetia illucens*) prepupae-based formulations with differentiated fatty acid profiles. Sci. Total Environ. 794, 148647. https://doi.org/10.1016/j.scitotenv.2021.148647

Jucker, C., Erba, D., Leonardi, M.G., Lupi, D. & Savoldelli, S., 2017. Assessment of vegetable and fruit substrates as potential rearing media for *Hermetia illucens* (Diptera: Stratiomyidae) larvae. Environ. Entomol. 46, 1415-1423. https://doi.org/10.1093/ee/nvx154

Kenar, J.A., Moser, B.R. & List, G.R., 2017. Chapter 2: Naturally occurring fatty acids: sources, chemistry, and uses. In: Fatty acids. 1st Edition. Ed: Ahmad, M.U., AOCS Press, London, United Kingdom. pp.24-71. https://doi.org/10.1016/B978-0-12-809521-8.00002-7

Liland, N.S., Biancarosa, I., Araujo, P, Biemans, D., Bruckner, C.G., Woogbø, R., Torstensen, B.E. & Lock, E.J., 2017. Modulation of nutrient composition of black soldier fly (*Hermetia illucens*) larvae by feeding seaweed-enriched media. PLoS One. 12. https://doi.org/10.1371/journal.pone.0183188

Meneguz, M., Schiavone, A., Gai, F., Dama, A., Lussiana, C., Renna, M. & Gasco, L., 2018. Effect of rearing substrate on growth performance, waste reduction efficiency and chemical composition of black soldier fly (*Hermetia illucens*) larvae. J. Sci. Food Agric. 98, 5776–5784. https://doi.org/10.1002/jsfa.9127

Stellenbosch University https://scholar.sun.ac.za

Oonincx, D.G.A.B., van Broekhoven, S., van Huis, A. & van Loon, J.J.A., 2015. Feed conversion, survival and development, and composition of four insect species on diets composed of food by-products. PLoS One. 10. https://doi.org/10.1371/journal.pone.0144601

Oonincx, D.G.A.B., Laurent, S., Veenenbos, M.E. & van Loon, J.J.A., 2020. Dietary enrichment of edible insects with omega 3 fatty acids. Iinsect Sci. 27, 500-509. https://doi.org/10.1111/1744-7917.12669

Romano, N., Fischer, H., Kumar, V., Francis, S.A. & Sinha, A.K., 2021. Productivity, conversion ability, and biochemical composition of black soldier fly (*Hermetia illucens*) larvae fed with sweet potato, spent coffee or dough. Int. J. Trop. Insect Sci. https://doi.org/10.1007/s42690-021-00532-5

Saadoun, J.H., Montevecchi, G., Zanasi, L., Bortolini, S., Macavei, L.I., Masino, F., Maistrello, L. & Antonelli, A., 2020. Lipid profile and growth of black soldier flies (*Hermetia illucens*, Stratiomyidae) reared on by-products from different food chains. J. Sci. Food Agric. 100, 3648-3657. https://doi.org/10.1002/jsfa.10397

Schreven, S.J.J., Yener, S., van Valenberg, H.J.F., Dicke, M. & van Loon, J.J.A., 2021. Life on a piece of cake: performance and fatty acid profiles of black soldier fly larvae fed oilseed by-products. J. Insects as Food Feed. 7, 35-49. https://doi.org/10.3920/JIFF2020.0004

Somroo, A.A., ur Rehman, K., Zheng, L., Cai, M., Xiao, X., Hu, S., Mathys, A., Gold, M., Yu, Z. & Zhang, J., 2019. Influence of Lactobacillus buchneri on soybean curd residue co-conversion by black soldier fly larvae (*Hermetia illucens*) for food and feedstock production. Waste Manag. 86, 114-122. https://doi.org/10.1016/j.wasman.2019.01.022

St-Hilaire, S., Cranfill, K., McGuire, M.A., Mosley, E.E., Tomberlin, J.K., Newton, L., Sealey, W., Sheppard, C. & Irving, S., 2007. Fish offal recycling by black soldier fly produces a foodstuff high in omega-3 fatty acids. J. World Aquac. Soc. 38, 197-202. https://doi.org/10.1111/j.1749-7345.2007.00101.x

Starcevic, K., Lozica, L., Gavrilovic, A., Heruc, Z. & Masek, T., 2019. Fatty acid plasticity of black soldier fly (*Hermetia Illucens*) larvae reared on alternative feeding media: crude olive cake and processed animal protein. J. Anim. Feed Sci. 28, 374-382. https://doi.org/10.22358/jafs/114434/2019

Truzzi, C., Giorgini, E., Annibaldi, A., Antonucci, M., Illuminati, S., Scarponi, G., Riolo, P., Isidoro, N., Conti, C., Zarantoniello, M., Cipriani, R. & Olivotto, I., 2020. Fatty acids profile of black soldier fly (*Hermetia illucens*): influence of feeding substrate based on coffee-waste silverskin enriched with microalgae. Anim. Feed Sci. Technol. 259, 114309. https://doi.org/10.1016/j.anifeedsci.2019.114309

Stellenbosch University https://scholar.sun.ac.za

Wang, S.Y., Wu, L., Li, B. & Zhang, D., 2020. Reproductive potential and nutritional composition of *Hermetia illucens* (Diptera: Stratiomyidae) prepupae reared on different organic wastes. J. Econ. Entomol. 113, 527-537. https://doi.org/10.1093/jee/toz296.

Stellenbosch University https://scholar.sun.ac.za

CHAPTER 7
Discussion

7.1 Publications

The primary objective of this study was to perform a systematic review of published research with the aim to determine the effect of rearing substrate composition on the fatty acid profile of the black soldier fly larvae (BSFL). Data were collected from each of the studies in an attempt to determine where the focus of present research lies and how it has developed and changed over the last decade. Meta-analyses were also performed as part of the systematic review to quantify the extent of the effect on the individual fatty acids using empirical data collected from the studies.

The oldest relevant study identified through the systematic review process was published in 2007 (St-Hilaire *et al.*, 2007). The research interest in BSFL and their potential in different industries has grown since then and it seems that the number of publications annually has increased with each consecutive year.

The three main areas of research that contributed to the collected publications were nutrition, waste management and biofuel production. Each one of the fields showed different research foci and aims. Nutrition research investigated how the use of specific substrates would improve the nutritional composition, especially the lipid content and fatty acid profile, of the BSFL. Studies either incorporated specific, sometimes unconventional, raw materials or by-products in the rearing substrates and evaluated the effect on the larval nutritional composition. Waste management research focused on the use of BSFL to manage and reduce various organic wastes. Numerous studies focused on both nutrition and waste management. Different waste products such as fish offal, oil cake and manure were used as rearing substrates and the effect on the larval nutritional composition was evaluated (St-Hilaire *et al.*, 2007; El-Dakar *et al.*, 2021a; Schreven *et al.*, 2021). The dual focused research thus investigated how BSFL could be utilised to develop a circular economy by addressing issues related to both nutrition and waste management. Less of the included publications focused on biofuel production. Those that were included did however add value to the collection of knowledge. The biofuel production publications tended to focus on different characteristics of the larval lipids. This is because the larval fatty acid profile that is desirable for biofuel production is different to larval fatty acid profile that nutrition-based research aims

Stellenbosch University https://scholar.sun.ac.za

to produce. The major difference is in terms of the ratio of saturated fatty acids (SFAs) to unsaturated fatty acids (UFAs). For biofuel production it is more suitable to use an oil that consists of a larger proportion of SFAs, specifically short and medium chain SFAs, as it decreases mechanical problems and risks of oxidation (Surendra *et al.*, 2016). For nutritional research the desired fatty acid profile varied depending on the intended animal that the larvae would be fed to, or if they were intended for human consumption. All the publications, irrespective of their research field, found that the rearing substrate composition had an effect on the composition of the larvae.

A number of more recently published studies investigated how the BSFL metabolic pathways function, focussing on the genes that are present in the larvae that contribute to the specific metabolic pathways that are involved in the synthesis, conversion and accumulation of specific fatty acids (Rabani *et al.*, 2019; Hoc *et al.*, 2020). As researchers' understanding of BSFL increase so has the complexity of the research methodologies and research foci.

As this study was focused on the fatty acid profile of the larvae, larval rearing trial details were collected from the included studies. It was found that there is still a large amount of variation when it comes to methodology details regarding the rearing. Variations such as total duration of the rearing trials, implementation of pre-harvest purging, killing method and life stage at harvest were encountered. Studies have indicated that methodological differences can influence the lipid content and the fatty acid profile of the larvae (Caligiani *et al.*, 2019; Giannetto *et al.*, 2020; Egnew *et al.*, 2021).

The aspect of the larval composition that was of major importance to this review was the fatty acid profile and specifically how it was affected by the rearing substrate. The BSFL fatty acid profile is clearly affected by the rearing substrate composition as this was found by all the publications that analysed the fatty acid composition. The specifics of the mechanisms involved and the extent to which each individual fatty acid is affected was however not always clarified by the publications. This indicated a gap in the knowledge regarding the BSFL fatty acid profile. The meta-analyses were performed with the aim on providing clarity regarding these questions.

Interestingly, less than half of the studies that performed rearing trials reported the proximate composition of the rearing substrate. Even fewer studies reported the rearing substrate fatty acid profile. There was however an increased tendency to perform and report these analyses in studies that were more recently published. This indicated a shift in research from only investigating the effect of specific raw materials in the rearing substrate to the chemical

Stellenbosch University https://scholar.sun.ac.za

and nutritional composition of the rearing substrate and how it translates into the larval fatty acid profile.

Through the examination and evaluation of the included publications, 26 studies (29 trials) were selected to be included in the meta-analysis aspect of this study. The meta-analysis results included the average intervention effect. This indicated the magnitude of the effect of the rearing substrate composition of the concentrations of individual fatty acids as well as the total amount of SFA, MUFA and PUFA. This provided a numeric measurement of the extent of change in fatty acid profile that would be achieved through rearing substrate composition changes. The meta-analysis results are shown in Table 7..

Table 7.1: Summary of meta-analysis statistical results

Fatty acid	**Average effect size (mean [CI])**	**Z-test P-value**	**Chi^2 P-value**	**Tau^2**	**I^2**
Saturated fatty acids (SFAs)					
Capric acid	0.06 [0.02, 0.10]	0.006	1.00	0.00	0%
Lauric acid	0.44 [0.32, 0.55]	< 0.00001	0.002	0.04	49%
Myristic acid	0.12 [0.08, 0.17]	< 0.00001	0.98	0.00	0%
Palmitic acid	0.13 [0.08, 0.19]	< 0.00001	0.99	0.00	0%
Margaric acid	0.04 [-0.02, 0.09]	0.17	1.00	0.00	0%
Stearic acid	0.12 [0.06, 0.17]	< 0.00001	0.07	0.01	29%
Arachidic acid	0.12 [-0.03, 0.27]	0.12	<0.00001	0.03	79%
Behenic acid	0.23 [-0.21, 0.67]	0.31	<0.00001	0.15	96%
Total SFA	0.35 [0.25, 0.45]	< 0.00001	0.12	0.01	27%
Monounsaturated fatty acids (MUFAs)					
Myristoleic acid	0.05 [-0.02, 0.11]	0.16	1.00	0.00	0%
Pentadecenoic acid	0.01 [-0.11, 0.12]	0.89	0.98	0.00	0%
Palmitoleic acid	0.14 [0.09, 0.18]	< 0.00001	0.51	0.00	0%
Heptadecenoic acid	0.05 [-0.08, 0.17]	0.47	0.99	0.00	0%
Oleic acid	0.20 [0.14, 0.26]	< 0.00001	0.26	0.00	14%

Stellenbosch University https://scholar.sun.ac.za

Table 7.1: Continued

Monounsaturated fatty acids (MUFAs)					
Vaccenic acid	0.07 [0.02, 0.12]	0.005	0.95	0.00	0%
Gondoic acid	0.05 [-0.01, 0.11]	0.09	0.87	0.00	0%
Total MUFA	0.26 [0.17, 0.35]	<0.00001	0.04	0.02	38%
Polyunsaturated fatty acids (PUFAs)					
Linoleic acid	0.25 [0.18, 0.33]	<0.00001	0.007	0.02	44%
α-Linolenic acid	0.18 [0.11, 0.25]	<0.00001	<0.00001	0.02	64%
γ-Linolenic acid	0.14 [0.04, 0.25]	0.007	0.42	0.00	0%
Stearidonic acid	0.10 [-0.01, 0.21]	0.08	0.87	0.00	0%
Eicosadienoic acid	0.02 [-0.09, 0.14]	0.73	0.99	0.00	0%
Arachidonic acid	0.10 [0.01, 0.19]	0.03	0.18	0.00	32%
Eicosapentaenoic acid	0.13 [0.03, 0.23]	0.01	0.83	0.00	0%
Docosapentaenoic acid	0.09 [-0.10, 0.28]	0.34	0.92	0.00	0%
Docohexaenoic acid	0.13 [0.02, 0.24]	0.03	0.87	0.00	0%
Total n-6	0.28 [0.08, 0.47]	0.006	0.03	0.03	59%
Total n-3	0.37 [0.23, 0.51]	<0.00001	0.06	0.02	51%
Total PUFA	0.33 [0.21, 0.44]	<0.00001	0.15	0.01	25%

As seen in the table, the total SFA, MUFA and PUFA concentrations were significantly affected by the rearing substrate. The SFA proportion exhibited the largest effect. The other two proportions also exhibited relatively large effects. This demonstrates that it is possible to manipulate the level of saturation of the BSFL fatty acid profile through nutritional changes in the rearing substrate. To better understand how these proportions are influenced, the effect on the individual fatty acids that contribute to the proportions should be considered. Based on the BSFL lipid metabolism pathways that are known, it seems that their lipid metabolism functions in a similar fashion to those of non-ruminant animals and thus the rearing substrates is a major factor influencing their fatty acid profile. The larvae have the ability to accumulate fatty acids acquired through their diet. They however can also biosynthesize a number SFAs from carbohydrates in their diet and the ability to convert fatty acids through desaturase and elongation enzyme reactions.

Stellenbosch University https://scholar.sun.ac.za

There were 24 fatty acids that were reported as present in the BSFL fatty acid profile in a sufficient number of studies for meta-analyses to be performed. Only six of these identified fatty acids were consistently present in the larval fatty acid profile. These fatty acids were lauric, myristic, palmitic, stearic, oleic and linoleic acid. It is suggestable that these fatty acids are therefore the most important to the larvae. All six of them were significantly affected by the rearing substrate composition.

7.2 Saturated fatty acids

Generally, the black soldier fly larval fatty acid profile consists mostly of SFAs. Saturated fatty acids can contribute up to 86% of the fatty acid profile (Danieli *et al.*, 2019). Adult black soldier flies do not have functional mouth parts for feeding. Therefore, they have to build up energy reserves during their developmental stages that can sustain them during pupation and adulthood. Saturated fatty acids are better suited for this function as they are less prone to oxidation than UFAs.

Lauric acid is arguably the most prominent SFA found in BSFL (C12:0). One study reported that the larval fatty acid profile could contain up to 68% lauric acid as a percentage of the total fatty acids, which was the highest concentration reported for any individual fatty acid in the larvae (Jucker *et al.*, 2017). Studies have suggested that the BSFL fatty acid profile contains more lauric acid compared to the fatty acid profiles of other insect species (Ewald *et al.*, 2020; Oonincx *et al.*, 2020). The meta-analysis performed on the lauric acid data in this review found that it was the fatty acid that exhibited the largest effect due to rearing substrate composition. Multiple studies stated that BSFL can contain relatively high proportions of lauric acid even if it is not present in the rearing substrate by using the available carbohydrates in the substrate to synthesize lauric acid (Spranghers *et al.*, 2017; Schreven *et al.*, 2021). It was suggested that the larval lauric acid content was increased when the larvae were reared on substrates that contained low concentrations of lipids. Its proportion to other SFAs was reduced when the concentration of the other SFAs were increased in the rearing substrate.

Besides lauric acid, the saturated fatty acid proportion of the larval fatty acid profile was found to largely consist of myristic (C14:0), palmitic (C16:0) and stearic acid (C18:0). All three of these SFA were significantly affected by the rearing substrate composition. The changes in concentrations were however smaller in magnitude than that found for lauric

Stellenbosch University https://scholar.sun.ac.za

acid. It seems that the larvae have the ability to both accumulate and synthesize these fatty acids (Hoc *et al.*, 2020).

Three of the SFA were not significantly affected by the rearing substrate composition (margaric, arachidic and behenic acid). It was hypothesized that the larvae have a limited ability to accumulate these fatty acids. Therefore, even if the fatty acids are found in high concentrations in the rearing substrate, they will mostly be metabolized or converted into other preferred fatty acids.

The publications included in this review generally reported if there were significant effects observed for one, generally lauric acid, or a few of the SFA. The specifics of how the proportions of the individual fatty acids were affected or why some were affected and others not was not widely discussed. This indicates potential for future research to investigate possible interactions between the carbohydrate content and the fatty acid profile of the rearing substrate in terms of the effect on the BSFL fatty acid profile.

7.3 Monounsaturated fatty acids

Monounsaturated fatty acids (MUFAs) make up the second largest proportion of the BSFL fatty acid profile after SFAs and the BSFL fatty acid profile was found to consist of up to 63% MUFA as a percentage of the total fatty acids (Schreven *et al.*, 2021). This is predominantly attributable to oleic acid (C18:1 cis-9). After lauric acid, oleic acid seems to be present in the highest concentrations in the BSFL fatty acid profile and was the only MUFA that was identified as consistently present in the BSFL fatty acid profile. The meta-analysis results indicated that oleic acid exhibited the largest effect, in other words change in concentration, due to rearing substrate composition among the MUFAs that were analysed.

Palmitoleic acid (C16:1 cis-9) is another main constituent of the MUFA present in the larval fatty acid profile and was identified in concentrations up to 15% of the total fatty acids in the larvae (El-Dakar *et al.*, 2021a). The meta-analysis of palmitoleic acid indicates that the rearing substrate composition had a significant effect on the palmitoleic acid concentration. The effect was however smaller than the effect observed on oleic acid. Vaccenic acid (C18:1 trans-11) and gondoic acid (C20:1 cis-11) concentrations were both found to be significantly affected by the rearing substrate composition. The larvae have the ability to both accumulate and biosynthesize some of the observed MUFA (Hoc *et al.*, 2020; Schreven *et al.*, 2021). It seems that their biosynthesis capacity differs for each of the MUFA.

Stellenbosch University https://scholar.sun.ac.za

Three of the MUFA were not significantly affected by the rearing substrate compositions. They were myristoleic acid (C14:1 n-5), pentadecenoic acid (C15:1 n-5) and heptadecenoic acid (C17:1 n-7). The highest concentrations observed for these MUFA in the BSFL fatty acid profile were lower than any of the other MUFA. They were also found to be frequently absent from the BSFL fatty acid profile. This may indicate that they are of less importance to the larvae and that they are only accumulated in small quantities. As previously stated, the larvae have the capability to biosynthesise MUFAs from SFAs. Therefore, it is likely that the effects observed on the SFA concentrations would also be reflected in the MUFA concentrations.

7.4 Polyunsaturated fatty acids

Polyunsaturated fatty acids (PUFAs) generally contribute a smaller proportion of the BSFL fatty acid compared to the SFAs and MUFAs. Multiple studies that were included in the systematic review focused on the concentration of PUFAs in the BSFL, specifically omega-3 fatty acids (Barroso *et al.*, 2019; Erbland *et al.*, 2020; Oonincx *et al.*, 2020; El-Dakar *et al.*, 2020, 2021b; Hoc *et al.*, 2021). The two PUFAs that are arguably the most important PUFAs in the BSFL fatty acid profile are linoleic acid (C18:2 n-6) and α-linolenic acid (C18:3 n-3). The meta-analysis results indicated that they were the PUFAs that experienced the largest significant effects due to the rearing substrate composition. Linoleic acid was however the only PUFA that was consistently present in the BSFL fatty acid profile. γ-Linolenic acid (C18:3 n-6), an isomer of linolenic acid also exhibited a relatively large effect. Eicosapentaenoic acid (C20:5 n-3) and docohexaenoic acid (C22:6 n-3) are also two PUFAs that are of importance in nutritional research due to their reported health benefits for humans and animals. Eicosapentaenoic and docohexaenoic acids were not always present in the larvae, but were found in relatively high concentrations in the larvae when they were present in the rearing substrate (Truzzi *et al.*, 2020). It appears that the larvae do not have the ability to synthesize these two PUFAs, but rather accumulate them directly from the rearing substrate.

The concentrations of the individual PUFAs also determine the omega-6 to omega-3 ratio of the BSFL fatty acid profile. The meta-analyses performed on the total omega-6 and total omega-3 content of the larvae also reported large significant effects due to the rearing substrate composition.

Stellenbosch University https://scholar.sun.ac.za

The meta-analyses suggested that three of the PUFAs were not significantly affected by the rearing substrate. These PUFAs were stearidonic acid (C18:4 n-3), eicosadienoic acid (C20:2 n-6) and docosapentaenoic acid (C22:5 n-6). All three of these PUFAs were reported as present in low concentrations in the BSFL.

7.5 Conclusion

The consolidated qualitative data collected from all the publications that were included in this study showed that there has been a great deal of progress in research surrounding the nutritional value of BSFL lipids and how their fatty acid profiles are affected by the composition of the rearing substrate. It does however also highlight the lack of standardisation regarding the larval rearing trial methodology and analysis reporting.

The meta-analysis results gave an indication of the most prominent fatty acids that are present in the BSFL and which fatty acids seem to invariably be present in the larvae. The relative extent to which the concentrations of the individual fatty acids could be manipulated through changes in the rearing substrate composition is awarded a level of clarity by the average effect sizes reported by the meta-analyses. It appears that the concentrations of the individual SFAs, notably lauric acid, are affected by the fatty acid profile and the carbohydrate content of the rearing substrate. The concentrations of the individual MUFAs and PUFAs are also affected by the fatty acid profile of the rearing substrate. More specifically an individual MUFA of PUFA concentrations in the BSFL will increase if it is present in higher concentrations in the rearing substrate. This was seen especially as pertaining to eicosapentaenoic acid and docohexaenoic acid.

These results, together with the collected data regarding the observed minimum and maximum concentrations of the individual fatty acids, could assist the development of more dynamic larval rearing substrate formulation. This could aid in the successful production of BSFL that have a fatty acid profile that is tailored to more closely meet the fatty acid requirements of their intended use. More research should however be done on how rearing substrate composition interacts with other factors, such as rearing environment and genetics, that affect the BSFL fatty acid profile.

The systematic review that was conducted for this study illustrated the extent of the research that has been done on the fatty acid profile of the BSFL. It also showed that there are still gaps in the knowledge regarding the larvae's ability to accumulate and synthesize the different fatty acids.

Stellenbosch University https://scholar.sun.ac.za

7.6 References

Barroso, F.G., Sánchez-Muros, M., Segura, M., Morote, E., Torres, A., Ramos, R. & Guil, J.L., 2017. Insects as food: enrichment of larvae of *Hermetia illucens* with omega 3 fatty acids by means of dietary modifications. J. Food Compos. Anal. 62, 8-13. https://doi.org/10.1016/j.jfca.2017.04.008

Caligiani, A., Marseglia, A., Sorci, A., Bonzanini, F., Lolli, V., Maistrello, L. & Sforza, S., 2019. Influence of the killing method of the black soldier fly on its lipid composition. Food Res. Int. 116, 276-282. https://doi.org/10.1016/j.foodres.2018.08.033

Danielie, P.P., Lussiana, C., Gasco, L., Amici, A. & Ronchi, B., 2019. The effect of diet formulation on the yield, proximate composition, and fatty acid profile of the black soldier fly (*Hermetia illucens* L.) prepupae intended for animal feed. Animals. 9, 178. doi:10.3390/ani9040178

Egnew, N., Romano, N., Fischer, H. & Sinha, A.K., 2021. Purging black soldier fly larvae (*Hermetia illucens*) compromises their nutritive value as a feedstuff. Int. J. Trop. Insect Sci. https://doi.org/10.1007/s42690-021-00491-x

El-Dakar, M.A., Ramzy, R.R. & Ji, H., 2021a. Influence of substrate inclusion of quail manure on the growth performance, body composition, fatty acid and amino acid profiles of black soldier fly larvae (*Hermetia illucens*). Sci. Total Environ. 772, 145528. https://doi.org/10.1016/j.scitotenv.2021.145528

El-Dakar, M.A., Ramzy, R.R., Ji, H. & Plath, M., 2020. Bioaccumulation of residual omega-3 fatty acids from industrial Schizochytrium microalgal waste using black soldier fly (*Hermetia illucens*) larvae. J. Clean. Prod. 268, 122288. https://doi.org/ 10.1016/j.jclepro.2020. 122288

El-Dakar, M.A., Ramzy, R.R., Wang, D. & Ji, H., 2021b. Sustainable management of Se-rich silkworm residuals by black soldier flies larvae to produce a high nutritional value and accumulate ω-3 PUFA. Waste Manag. 124, 72-81. https://doi.org/10.1016/ j.wasman.2021.01.040

Erbland, P., Alyokhin, A., Perkins, L.B. & Peterson, M., 2020. Dose-dependent retention of omega-3 fatty acids by black soldier fly larvae (Diptera: Stratiomyidae). J. Econ. Entomol. 113, 1221-1226. https://doi.org/10.1093/jee/toaa045

Ewald, N., Vidakovic, A., Langeland, M., Kiessling, A., Sampels, S. & Lalander, C., 2020. Fatty acid composition of black soldier fly larvae (*Hermetia illucens*) - possibilities and limitations for modification through diet. Waste Manag. 102, 40-47 https://doi.org/ 10.1016/j.wasman.2019.10.014

Stellenbosch University https://scholar.sun.ac.za

Giannetto, A., Oliva, S., Ceccon Lanes, C.F., de Araújo Pedron, F., Savastano, D., Baviera, C., Parrino, V., Lo Paro, G., Spano, N.C., Cappello, T., Maisano, M., Mauceri, A. & Fasulo, S., 2020. *Hermetia illucens* (Diptera: Stratiomydae) larvae and prepupae: biomass production, fatty acid profile and expression of key genes involved in lipid metabolism. J. Biotechnol. 307, 44-54 https://doi.org/10.1016/j.jbiotec.2019.10.015

Hoc, B., Francis, F., Carpentier, J., Mostade, L., Blecker, C., Purcaro, G. & Caparros Megido, R., 2021. ω3-enrichment of *Hermetia illucens* (L. 1758) prepupae from oilseed byproducts. J. Saudi Soc. Agric. Sci. 20, 155-163. https://doi.org/10.1016/j.jssas.2021.01.001

Hoc, B., Genva, M., Fauconnier, M-L., Lognay, G., Francis, F. & Megido, R.C., 2020. About lipid metabolism in *Hermetia illucens* (L. 1758): on the origin of fatty acids in prepupae. Sci. Rep. 10, 11916. https://doi.org/10.1038/s41598-020-68784-8

Jucker, C., Erba, D., Leonardi, M.G., Lupi, D. & Savoldelli, S., 2017. Assessment of vegetable and fruit substrates as potential rearing media for *Hermetia illucens* (Diptera: Stratiomyidae) larvae. Environ. Entomol. 46, 1415-1423. https://doi.org/10.1093/ee/nvx154

Oonincx, D.G.A.B., Laurent, S., Veenenbos, M.E. & van Loon, J.J.A., 2020. Dietary enrichment of edible insects with omega 3 fatty acids. Iinsect Sci. 27, 500-509. https://doi.org/10.1111/1744-7917.12669

Rabani, V., Cheatsazan, H. & Davani, S., 2019. Proteomics and lipidomics of black soldier fly (Diptera: Stratiomyidae) and blow fly (Diptera: Calliphoridae) larvae. J. Insect Sci. 19, 29. https://doi.org/10.1093/jisesa/iez050

Schreven, S.J.J., Yener, S., van Valenberg, H.J.F., Dicke, M. & van Loon, J.J.A., 2021. Life on a piece of cake: performance and fatty acid profiles of black soldier fly larvae fed oilseed by-products. J. Insects as Food Feed. 7, 35-49. https://doi.org/10.3920/JIFF2020.0004

Spranghers, T., Ottoboni, M., Klootwijk, C., Ovyn, A., Deboosere, S., De Meulenaer, B., Michiels, J., Eeckhout, M., De Clercq, P. & De Smet, S., 2017. Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates. J. Sci. Food Agric. 97, 2594-2600. https://doi.org/10.1002/jsfa.8081

St-Hilaire, S., Cranfill, K., McGuire, M.A., Mosley, E.E., Tomberlin, J.K., Newton, L., Sealey, W., Sheppard, C. & Irving, S., 2007. Fish offal recycling by black soldier fly produces a foodstuff high in omega-3 fatty acids. J. World Aquac. Soc. 38, 197-202. https://doi.org/10.1111/j.1749-7345.2007.00101.x

Stellenbosch University https://scholar.sun.ac.za

Starcevic, K., Lozica, L., Gavrilovic, A., Heruc, Z. & Masek, T., 2019. Fatty acid plasticity of black soldier fly (*Hermetia Illucens*) larvae reared on alternative feeding media: crude olive cake and processed animal protein. J. Anim. Feed Sci. 28, 374-382. https://doi.org/10.22358/jafs/114434/2019

Surendra, K.C., Olivier, R., Tomberlin, J.K., Jha, R. & Khanal, S.K., 2016. Bioconversion of organic wastes into biodiesel and animal feed via insect farming. Renew. Energy. 98, 197-202. https://doi.org/10.1016/j.renene.2016.03.022

Truzzi, C., Giorgini, E., Annibaldi, A., Antonucci, M., Illuminati, S., Scarponi, G., Riolo, P., Isidoro, N., Conti, C., Zarantoniello, M., Cipriani, R. & Olivotto, I., 2020. Fatty acids profile of black soldier fly (*Hermetia illucens*): influence of feeding substrate based on coffee-waste silverskin enriched with microalgae. Anim. Feed Sci. Technol. 259, 114309. https://doi.org/10.1016/j.anifeedsci.2019.114309

Stellenbosch University https://scholar.sun.ac.za

APPENDIX A

Table A 1: List of included studies and indication of meta-analysis inclusion

Study reference	Inclusion in meta-analyses
(Abduh *et al.*, 2018)	No
(Alifian *et al.*, 2019)	No
(Barbi *et al.*, 2020)	No
(Barragan-Fonseca *et al.*, 2017)	No
(Barroso *et al.*, 2017)	Yes
(Barroso *et al.*, 2019)	Yes
(Bbosa *et al.*, 2019)	No
(Benzertiha *et al.*, 2020)	No
(Caligiani *et al.*, 2018)	No
(Caligiani *et al.*, 2019)	No
(Campbell *et al.*, 2020)	No
(Cullere *et al.*, 2019)	No
(Danieli *et al.*, 2019)	Yes
(Egnew *et al.*, 2021)	No
(El-Dakar *et al.*, 2020)	No
(El-Dakar *et al.*, 2021b)	Yes
(El-Dakar *et al.*, 2021a)	Yes
(El-Dakar *et al.*, 2021c)	Yes
(Erbland *et al.*, 2020)	No
(Ewald *et al.*, 2020)	Yes
(Fischer *et al.*, 2021)	Yes
(Galassi *et al.*, 2021)	Yes
(Gao *et al.*, 2019)	Yes

Stellenbosch University https://scholar.sun.ac.za

Table A 1: Continued

Study reference	Inclusion in meta-analyses
(Giannetto *et al.*, 2020)	No
(Grossule *et al.*, 2020)	No
(Guil-Guerrero *et al.*, 2020)	Yes
(Hoc *et al.*, 2020)	No
(Hoc *et al.*, 2021a)	Yes
(Hoc *et al.*, 2021b)	Yes
(Jucker *et al.*, 2017)	Yes
(Jucker *et al.*, 2020)	No
(Kawasaki *et al.*, 2019)	No
(Kieronczyk *et al.*, 2020)	No
(Koutsos *et al.*, 2019)	No
(Kuppusamy *et al.*, 2020)	No
(Leong *et al.*, 2015)	No
(Leong & Kutty, 2020b)	No
(Leong & Kutty, 2020a)	No
(Liland *et al.*, 2017)	Yes
(Liu *et al.*, 2017)	No
(Liu *et al.*, 2018)	No
(Matthaeus *et al.*, 2019)	No
(Meneguz *et al.*, 2018)	Yes
(Mohamad *et al.*, 2020)	No
(Mohd-Noor *et al.*, 2017)	No
(Nguyen *et al.*, 2015)	No
(Nyakeri *et al.*, 2017)	No

Stellenbosch University https://scholar.sun.ac.za

Table A 1: Continued

Study reference	Inclusion in meta-analyses
(Oonincx *et al.*, 2015)	Yes
(Oonincx *et al.*, 2020)	Yes
(Opatovsky *et al.*, 2021)	No
(Pang *et al.*, 2020)	No
(Pastor *et al.*, 2015)	No
(Rabani *et al.*, 2019)	No
(Riudavets *et al.*, 2020)	No
(Romano *et al.*, 2021)	Yes
(Saadoun *et al.*, 2020)	Yes
(Scala *et al.*, 2020)	No
(Schreven *et al.*, 2021)	Yes
(Shumo *et al.*, 2019)	No
(Somroo *et al.*, 2019)	Yes
(Spranghers *et al.*, 2017)	No
(Starcevic *et al.*, 2019)	Yes
(St-Hilaire *et al.*, 2007)	Yes
(Surendra *et al.*, 2016)	No
(Surendra *et al.*, 2020)	No
(Truzzi *et al.*, 2020)	Yes
(Wang *et al.*, 2020)	Yes
(Weru *et al.*, 2021)	No
(Wong *et al.*, 2019)	No

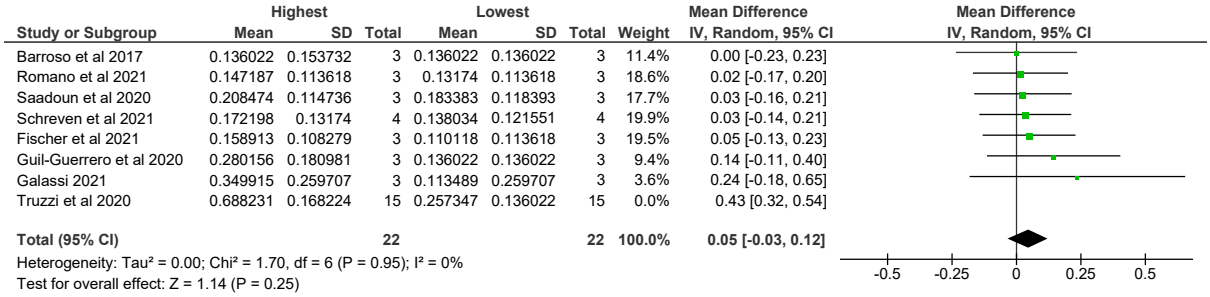
Stellenbosch University https://scholar.sun.ac.za

Table A 2: List of studies included in meta-analyses

Studies included in meta-analyses
(Barroso *et al.*, 2017)
(Barroso *et al.*, 2019)
(Danieli *et al.*, 2019)
(El-Dakar *et al.*, 2020)
(El-Dakar *et al.*, 2021a)
(El-Dakar *et al.*, 2021b)
(Ewald *et al.*, 2020)
(Fischer *et al.*, 2021)
(Galassi *et al.*, 2021)
(Gao *et al.*, 2019)
(Guil-Guerrero *et al.*, 2020)
(Hoc *et al.*, 2021a)
(Hoc *et al.*, 2021b)
(Jucker *et al.*, 2017)
(Liland *et al.*, 2017)
(Meneguz *et al.*, 2018)
(Oonincx *et al.*, 2015)
(Oonincx *et al.*, 2020)
(Romano *et al.*, 2021)
(Saadoun *et al.*, 2020)
(Schreven *et al.*, 2021)
(Somroo *et al.*, 2019)
(Starcevic *et al.*, 2019)
(St-Hilaire *et al.*, 2007)
(Truzzi *et al.*, 2020)
(Wang *et al.*, 2020)

Stellenbosch University https://scholar.sun.ac.za

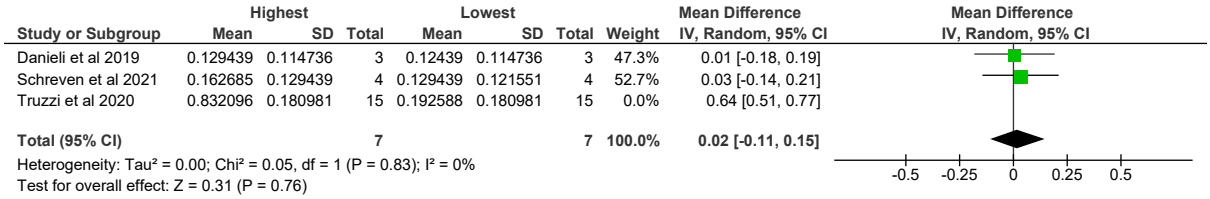
APPENDIX B



Study or Subgroup	Highest Mean	Highest SD	Highest Total	Lowest Mean	Lowest SD	Lowest Total	Weight	Mean Difference IV, Random, 95% CI
Barroso et al 2017	0.136022	0.153732	3	0.136022	0.136022	3	11.4%	0.00 [-0.23, 0.23]
Romano et al 2021	0.147187	0.113618	3	0.13174	0.113618	3	18.6%	0.02 [-0.17, 0.20]
Saadoun et al 2020	0.208474	0.114736	3	0.183383	0.118393	3	17.7%	0.03 [-0.16, 0.21]
Schreven et al 2021	0.172198	0.13174	4	0.138034	0.121551	4	19.9%	0.03 [-0.14, 0.21]
Fischer et al 2021	0.158913	0.108279	3	0.110118	0.113618	3	19.5%	0.05 [-0.13, 0.23]
Guil-Guerrero et al 2020	0.280156	0.180981	3	0.136022	0.136022	3	9.4%	0.14 [-0.11, 0.40]
Galassi 2021	0.349915	0.259707	3	0.113489	0.259707	3	3.6%	0.24 [-0.18, 0.65]
Truzzi et al 2020	0.688231	0.168224	15	0.257347	0.136022	15	0.0%	0.43 [0.32, 0.54]
Total (95% CI)			**22**			**22**	**100.0%**	**0.05 [-0.03, 0.12]**

Heterogeneity: Tau² = 0.00; Chi² = 1.70, df = 6 (P = 0.95); I² = 0%
Test for overall effect: Z = 1.14 (P = 0.25)

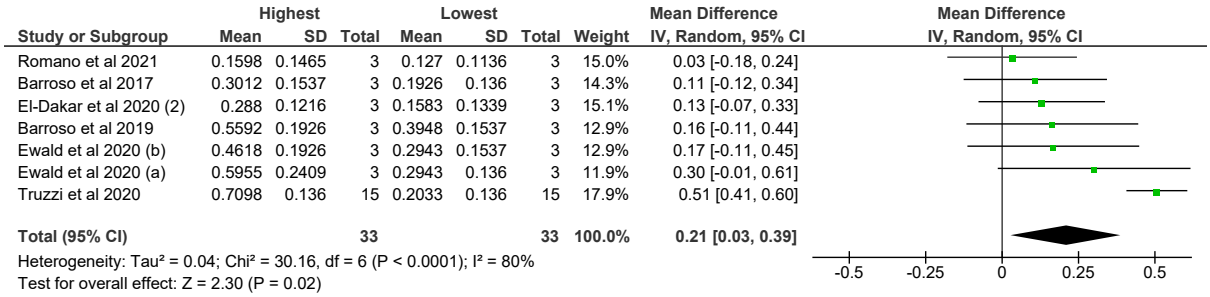
Figure B 1: Software output of second meta-analysis of arachidic acid concentration of black soldier fly larval fatty acid profile



Study or Subgroup	Highest Mean	Highest SD	Highest Total	Lowest Mean	Lowest SD	Lowest Total	Weight	Mean Difference IV, Random, 95% CI
Danieli et al 2019	0.129439	0.114736	3	0.12439	0.114736	3	47.3%	0.01 [-0.18, 0.19]
Schreven et al 2021	0.162685	0.129439	4	0.129439	0.121551	4	52.7%	0.03 [-0.14, 0.21]
Truzzi et al 2020	0.832096	0.180981	15	0.192588	0.180981	15	0.0%	0.64 [0.51, 0.77]
Total (95% CI)			**7**			**7**	**100.0%**	**0.02 [-0.11, 0.15]**

Heterogeneity: Tau² = 0.00; Chi² = 0.05, df = 1 (P = 0.83); I² = 0%
Test for overall effect: Z = 0.31 (P = 0.76)

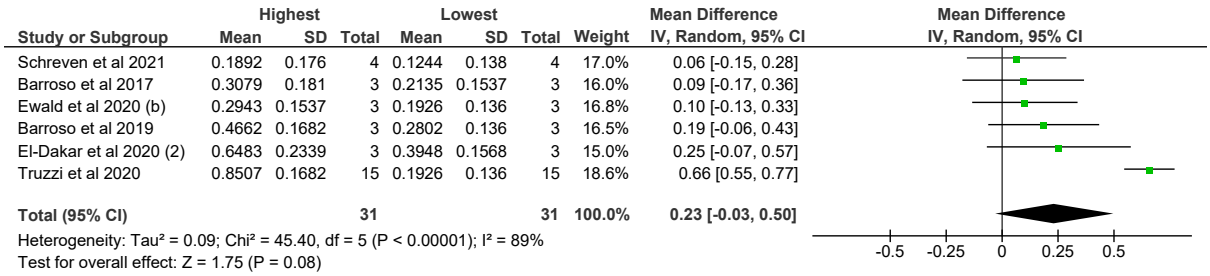
Figure B 2: Software output of second meta-analysis of behenic acid concentration of black soldier fly larval fatty acid profile



Study or Subgroup	Highest Mean	Highest SD	Highest Total	Lowest Mean	Lowest SD	Lowest Total	Weight	Mean Difference IV, Random, 95% CI
Romano et al 2021	0.1598	0.1465	3	0.127	0.1136	3	15.0%	0.03 [-0.18, 0.24]
Barroso et al 2017	0.3012	0.1537	3	0.1926	0.136	3	14.3%	0.11 [-0.12, 0.34]
El-Dakar et al 2020 (2)	0.288	0.1216	3	0.1583	0.1339	3	15.1%	0.13 [-0.07, 0.33]
Barroso et al 2019	0.5592	0.1926	3	0.3948	0.1537	3	12.9%	0.16 [-0.11, 0.44]
Ewald et al 2020 (b)	0.4618	0.1926	3	0.2943	0.1537	3	12.9%	0.17 [-0.11, 0.45]
Ewald et al 2020 (a)	0.5955	0.2409	3	0.2943	0.136	3	11.9%	0.30 [-0.01, 0.61]
Truzzi et al 2020	0.7098	0.136	15	0.2033	0.136	15	17.9%	0.51 [0.41, 0.60]
Total (95% CI)			**33**			**33**	**100.0%**	**0.21 [0.03, 0.39]**

Heterogeneity: Tau² = 0.04; Chi² = 30.16, df = 6 (P < 0.0001); I² = 80%
Test for overall effect: Z = 2.30 (P = 0.02)

Figure B 3: Software output of preliminary meta-analysis of eicosapentaenoic acid concentration of black soldier fly larval fatty acid profile



Study or Subgroup	Highest Mean	Highest SD	Highest Total	Lowest Mean	Lowest SD	Lowest Total	Weight	Mean Difference IV, Random, 95% CI
Schreven et al 2021	0.1892	0.176	4	0.1244	0.138	4	17.0%	0.06 [-0.15, 0.28]
Barroso et al 2017	0.3079	0.181	3	0.2135	0.1537	3	16.0%	0.09 [-0.17, 0.36]
Ewald et al 2020 (b)	0.2943	0.1537	3	0.1926	0.136	3	16.8%	0.10 [-0.13, 0.33]
Barroso et al 2019	0.4662	0.1682	3	0.2802	0.136	3	16.5%	0.19 [-0.06, 0.43]
El-Dakar et al 2020 (2)	0.6483	0.2339	3	0.3948	0.1568	3	15.0%	0.25 [-0.07, 0.57]
Truzzi et al 2020	0.8507	0.1682	15	0.1926	0.136	15	18.6%	0.66 [0.55, 0.77]
Total (95% CI)			**31**			**31**	**100.0%**	**0.23 [-0.03, 0.50]**

Heterogeneity: Tau² = 0.09; Chi² = 45.40, df = 5 (P < 0.00001); I² = 89%
Test for overall effect: Z = 1.75 (P = 0.08)

Figure B 4: Software output of preliminary meta-analysis of docohexaenoic acid concentration of black soldier fly larval fatty acid profile